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ON A FUNGI-DISEASE OF EEL

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(Received Dec. 15, 1955)

A disease of eel so-called "Sure" among Japanese eel-culturists in Kawajiri district of Shizuoka Pref. is often observable from spring into early summer in eel-culture ponds.

In 1955, the severe prevalence of the disease broke out and caused extensive losses of eels especially in the above-mentioned district.

The mortality of affected fish is considerably high, so the disease appears to be a significant problem in eel-cultural management. The present paper deals with some observations on the disease in special references to *Saprolegnia parasitica* Coker which may be a causative agent of the disease.

Before going further we wish to acknowledge our indebtedness to Messrs. Yasuo Sugiura, Tokuo Sano and Minoru Nomura of Tokyo University of Fisheries and Dr. Keishi Amano of Tokai Regional Fisheries Institute and Dr. Prof. Yasuo Ôshima of Tokyo University for their aids in various ways.

Further we thank to Messrs. Keijiro Muramatsu, Chief director of Fish Cultural Fisheries Co-operative Association of Lake Hamana, and Kyo Kubota, that of Haibara, and Shiro Suzuki, Managing director of Tokyo Fresh-Water Fish Association for their aids in the supply of materials necessary to the present study.

Lastly, we must express our hearty thanks to the Ministry of Education for the expense of present study, partly defrayed by the Grant in Aid for Experimental Scientific Research.

1. Epidemic time, mortality and symptom

The epidemic was observed during early March and early May and continued about 3 weeks on the average. And the disease ceased immediately after the water temperature became stable and went up to 18°C. The water temperatures showed 14~21°C, on average 16.6°C, during the epidemics.

Table 1 shows the time when the disease broke out, observed in 39 eel-culture ponds.

The loss of fish in each pond was 2–70% of the population of fish under cultivation. The total mortality among 81,442 Kilograms eels was 19.4%.

The disease attacks eels of all classes under cultivation during the season and out of relation to age or size of fish.

The disease is characterized by rapidity of the course. The diseased fish showed anorexia and they were swimming about inactively on water surface without crowding at the feeding place and gradually grew weak and fell dead. However, the natural recovery from the disease was observed at the respectable rate.

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Table 1. The time of outbreak of disease.

Time \ Items	Frequency	Percentage
March—		
First decade	2	4.8
Middle 〃	3	7.1
Last 〃	3	7.1
April—		
First decade	9	21.4
Middle 〃	10	23.8
Last 〃	10	23.8
May—		
First decade	5	11.9

Remark: Three cases of recrudescence are contained in frequency.

2. Findings at autopsy and cause of disease

Diseased fish showed a growth of fungi on the body surface especially at the snout or the tail portion. The affected parts resulted in necrosis and the skin became gray white or exfoliated and hemorrhagic ulcer developed in the later stages (Pl. I, Fig. 1). Under the microscope, the affected parts showed only necrotic figures in various degrees.

Generally, the epidermal tissue turned in dirty debris densely covered with the fungi or entirely fall out and the corium ruptured and dissolved in later stages. (Pl. I, Fig. 2). The ulcerous lesions exposed musculature had also the surface growth of fungi and it was observed that a part of mycelia infiltrated into musculature. The muscular tissue hemorrhagied and disintegrated partially. Some fish showed congestion in pectral fins or anal fin and, at the base. Excepting the liver and intestine, the internal organs appeared nearly normal. The liver and intestine showed inflammation generally.

The stomach and intestine contained no foods. The appearances of gills and heart blood were normal. As mentioned above, the most remarkable character of the diseased fish was the fact that they showed the growth of fungi on their surfaces.

However on account of very high mortality of affected fish, it seems doubtful whether the fungus is a primary cause of the disease. To clear this point an inoculation experiment was tried by the following method. The viscera obtained from five fish showing the typical sign of the disease were crushed in a mortar and ten times of distilled water of the quantity was added to it, then the fluid was filtered by Berkfeld's filter.

Six healthy eels weighting 60–105 g were injected in the body musculatures with 1.0 cc of the filtrate. For the control, same number of healthy ells were inoculated with 1.0 cc of the heated filtrate at 60°C for 60 minutes. The results of these experiments were negative and all the inoculated fish survived without showing any sign of the disease (Date of the experiments: May 18–30, '55, water temperature 18–20°C).

Therefore, the disease probably is not of virus nature.

Next, isolation of bacteria from affected fish was tested by using five

affected fish. And cultures of bacteria from heart blood, liver and kidney in one fish and from kidney in another fish were obtained. The cultures obtained from kidney of the former were mixed and other cultures were pure. One strain of bacteria (isolated from heart blood, liver and kidney of one fish and from kidney of another fish) was identical with *Pseudomonas punctata* f. *sacrowiensis* SCHÄPERCLAUS 1930, a causative bacteria of "Süsswasseraalrotseuche" and another strain was also a *Pseudomomas* group.

Consequently, it is obvious that some groups among diseased fish were infected with bacteria. On the other hand, it was proved that by the inoculation experiment with the fungus the heavy fungual infection was lethal to eels.

From the fact, it is conjectured that the main cause of the mortality may still be attributable to the fungus.

3. Isolation of fungus

Small pieces of debris from the lesion of the diseased fish captured at an eel-culture pond in Kawajiri district of Shizuoka Pref. on May 14, '55 were cultured on Bouillon agar-plates at room temperatures.

All the cultures showed the growth of a strain of fungus, which was identical with *Saprolegnia parasitica* COKER. After successive subcultures on Bouillon agar-plate, on which a small quantity of penicillin and streptomycin were spread, thus cultures free from bacteria of the fungus were obtained. Although the culture showed luxuriant growth of the fungus, yet ripening of sporangium was not seen. But, when the fungus was on transplanted simple agar-plate (Agar 0.15 g, Distilled water 10 cc), numerous zoospores were produced after 24 hours (Pl. I, Fig. 4). The zoospores were smeared on agar-plate, and the pure cultures of the fungus originating from the zoospores were obtained. For the subsequent study, the strain was used.

4. Characterstics of the fungus, *Saprolegnia parasitica* Coker

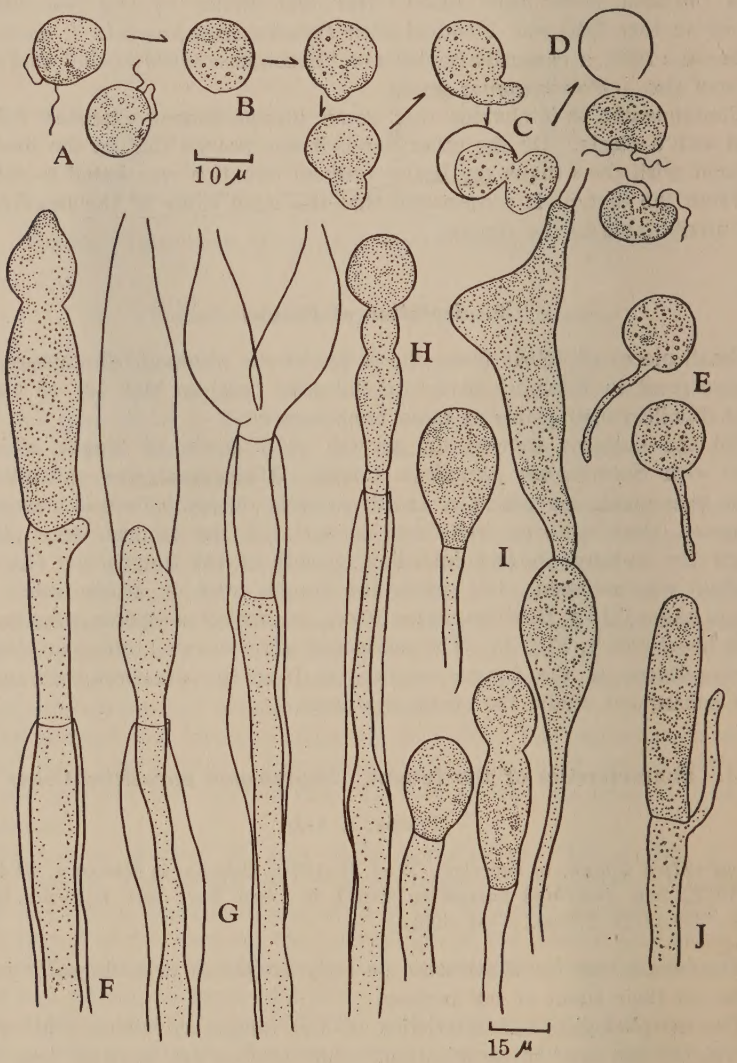
(Textfig. 1, A-J)

S. parasitica COKER, in Saproleg., 57 pl. 18 (1923); Ookubo, in NAGAO 4: 54 Fig. 43 (1953). Syn. *Isoachlya parasitica* NAGAI, in Journ. Fac. Agri. Hokkaido Imp. Univ., 32: 12 Pl. 2 Figs. 27–34 (1931).

The fungus was found parasitic on body surface of eels, *Anguilla japonica* especially on their snout or tail portion.

The morphological characteristics of the fungus as follows:—Mycelia are well developed but poor in ramification. Main hyphae are more or less 20μ in diameter, rami 10–20μ in diameter. Zoosporangia are spindly or clavated or of somewhat irregular form; 15–45μ × 50–400μ in size; and contain numerous zoospores. Secondary zoosporangium arises from by proliferation or sometimes lateral branching below the primary zoosporangium. Each primary zoospore swims out by himself from an apical pore of zoosporangium and it is elliptical just after being swum out but in about 30 seconds it becomes spherical with two

flagella at tip. The motility of zoospores is not active. For a while, the zoospor encysts and later on, a secondary zoospore which is reniform and with tw



Textfig. 1. *Saprolegnia parasitica* COKER.

A, primary zoospore; B, resting zoospore; C, formation of secondary zoospore; D, emptied zoospore; E, germination of secondary zoospore; F, primary zoosporangium and young secondary zoosporangium; G, secondary zoosporangium; H, primary zoosporangium and gemma; I~J, gemma.

flagella at side, escapes from it. Primary and secondary zoospores are same in size and measure 10 to 20μ in diameter.

Many gemmae are seen. They are clavate, spherical, ovoid or of chained form and occur at the end of mycelia or sometimes pleurogenous. Sexual phase is unknown.

Some observations on the fungus:—The mycelia are well stainable to carbolfuchsin or malachite green solution. Protoplasm contains many small globuls which are stainable with Sudan III. Gram staining is negative. Catalase reaction is positive. The fungus slowly grows at 4°C, rapidly at 28°C, ceases to grow at 37°C, but survives within 24 hours. Killed by drying in desiccator under low pressure within 24 hours. Killed by freezing at −4°C in 24 hours. Killed by 50°C for one minute.

5. Some biological studies on the fungus

(1) Cultures on various media.

Experimental—The fungus cultured on Bouillon-agar-plate at 23°C for 24 hours was cut out about 25 mm² in size and it was transferred on each medium. After cultured at 23°C for 24 or 48 hours, fungus grown area was measured.

Table 2. Growth of the fungus on various media.

Media	Composition		Area covered by the mycelia	
			after 24 h.	after 48 h.
Simple-Agar	Agar H_2O	15g 1000cc	6.6 cm²	38.5 cm²
Otmeal	Otmeal Agar H_2O	7g 15g 1000cc	3.8 cm²	25.8 cm²
Milk	Milk Agar H_2O	10cc 0.15g 10cc	10.2 cm²	43.2 cm²
Potato	Potato H_2O	20g 1cc	1.8 cm²	10.8 cm²
Soybeans-Extract	Soybeans Agar H_2O	150g 15g 1000cc	3.5 cm²	27.0 cm²
Carrot-Extract	Carrot Agar H_2O	250g 15g 1000cc	4.0 cm²	26.5 cm²
Eel flesh-Extract	Eel flesh Pepton Agar H_2O	150g 10g 15g 1000cc	16.6 cm²	28.3 cm²
Bouillon	Beef extract Pepton Agar H_2O	7g 10g 15g 1000cc	13.2 cm²	27.5 cm²
Eel-Blood	Eel-blood Agar H_2O	1cc 0.15g 9cc	11.3 cm²	23.7 cm²

Petri-dishes with 80mm in diameter and containing 10cc of each medium except ing potato were used for the culture. Each medium was adjusted pH 7.0.

Result—Results obtained are shown in Table 2.

As shown in Table 2, it is possible to culture the fungus on almost al kinds of media. The eel-flesh-agar and Bouillon-agar yielded excellent growth o fungus and the simple agar media was most inferior.

Morphologically, there was no differences among the fungi grown on eacl medium except the thickness or ramification of the mycelia. The grown fung on nutritious media such as eel-flesh-agar or Bouillon-agar were very dense an the mycelia thick and their ramification luxuriant. The fungus grown on carrot extract produced more gemmae than on other media. On eel-blood-agar, a pal green color appeared in the area covered by the mycelia. Probably, it originate from the formation of metro-haemoglobin.

(2) Resistance of the fungus against some disinfectants.

Experimental—The fungus cultured on Bouillon-agar-plate (at 23°C for 2 hours) was cut out into small pieces of about 25 mm^2 in size and the pieces were steeped in the disinfectants of 10 cc for the definite time and after washed b sterilized water, subcultures of them were made. The temperature of the dis infectants was kept at 20°C during the experiments.

Results—Are shown in Table 3.

Table 3. Resistance of the fungus against some disinfectants.

Disinfectant	Concentration	Steeped time and the effect
Malachite green	1: 20,000	Barely escaped dying 5 to 15 second after, died 20 seconds after.
	1: 30,000	Survived for 10 to 20 seconds; and died for 30 seconds after.
	1: 40,000	Died 40 seconds after.
	1: 50,000	Died 90 seconds after.
$KMnO_4$	1: 10,000	Almost no effect 5 to 15 minutes after; almost lethal 20 to 30 minutes after; died 40 minutes after.
	1: 20,000	Still survived for 60 minutes.
Formalin	1: 11,000	Survived 1 to 3 minutes after; died 4 minutes after.
	1: 2,000	Survived 5 to 10 mlnutes after; died 15 minutes after.
	1: 3,000	Almost no effect 5 to 20 minutes after; very few mycelia survived 30 minutes after; died 40 minutes after.
$CuSO_45H_2O$	1: 1,000	Almost no effect 5 to 20 minutes after; very few mycelia survived 50 minutes after; died 60 minutes after.
$Ca(OH)_2$	1: 100	No effect 1 to 5 minutes; almost lethal 10 minutes; died 15 minutes after.
Alcohol	70%	No effect 1 to 3 minutes; died 5 minutes after.

As shown in Table 3, the fungi were killed completely as follows: 1:20,000-1:50,000 malachite green solution for 20–90 seconds, 1:10,000 $KMnO_4$ solution fo 40 minutes; 1:1,000–1:3,000 formalin solution for 4–40 minutes; 1:1,000 $CuSO_45H_2$

solution for 60 minutes; 1:100 $Ca(OH)_2$ solution for 15 minutes and 70% alcohol for 5 minutes. Malachite green was the most toxic to the fungi among the disinfectants. Potassium permangnate and formalin also were powerful to kill the fungi.

(3) Resistance of the fungus against NaCl and malachite green solution.

Experimental—As in the preceding experiments, the fungus was cut out in size of 25 mm^2 from the Bouillon-agar-plate culture at room temperature for 24 hours and it was transplated in Bouillon-agar-plate containing NaCl or malachite green and after cultured for just 24 and 48 hours, the fungus grown area was measured.

Results—The experimental results are shown in Tables 4 to 5 and Textfig. 2.

Table 4. Resistance of the fungus against NaCl.

Concentration*	Growth of the fungus		Remark
	after 24 hours	after 48 hours	
0 %	10.39 cm^2	25.46 cm^2	
0.5	8.54	16.61	
1.0	6.15	13.85	
1.5	4.18	10.13	
2.0	2.93	8.04	
2.5	0.34	3.14	
3.0	0	0	Few mycelia develop in subculture.
3.5	0	0	
4.0	0	0	Subculture showed no growth of the fungus.

* 10cc medium used for the experiment; 0.5% = 0.5g NaCl in 10cc medium and so forth.

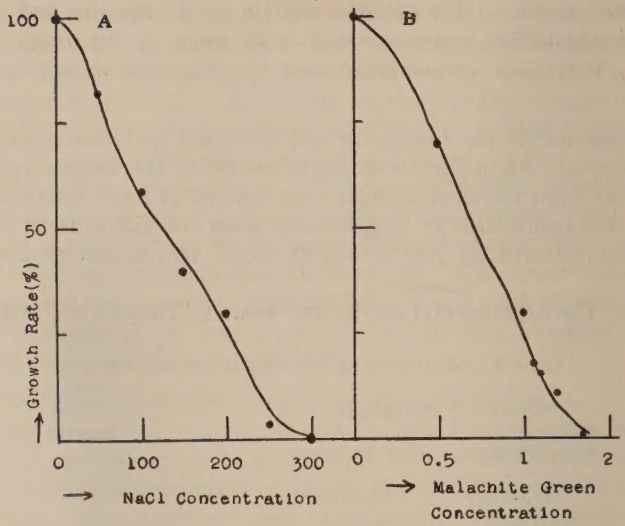
Table 5. Resistance of the fungus against malachite green.

Concentration*	Growth of the fungus	
	after 24 hours	after 48 hours
0	10.53 cm^2	24.97 cm^2
1: 200,000	7.35	19.63
1: 100,000	3.14	12.56
1: 90,000	1.39	11.93
1: 80,000	1.77	10.17
1: 70,000	1.13	8.13
1: 60,000	0	0
1: 50,000	0	0

* 0.1cc of each diluted malachite green solution added to the 10cc medium.

(4) Resistance of the fungus against bactericidal lamp radiation.

Experimental—A preliminary experiment was tried by radiating the bactericidal lamp-ray (wave length 2537Å, 800 microwatt/cm^2 at 20 cm distance) to the



Textfig. 2. A, Resistance of the fungus against NaCl; NaCl concentration= mg in 10cc medium; growth rate=relative growth compared with the growth of control with determined as 100% growth; B, Resistance of the fungus against malachite green; malachite green concentration=γ in 10 cc medium; growth rate =relative growth compared with the growth of control with determined as 100% growth.

cultured fungi (on Bouillon-agar-plate, at room temperature for 24 hours) at 20cm distance from the source for 5 to 30 minutes with 5 minutes intervals.

The experimental results hardly showed any effect within the treated time. So in the next experiment (Exp. 1), the fungus was exposed to the ray for 40 to 120 minutes with 10 or 30 minutes intervals (The experimental conditions were same as in preceding experiment).

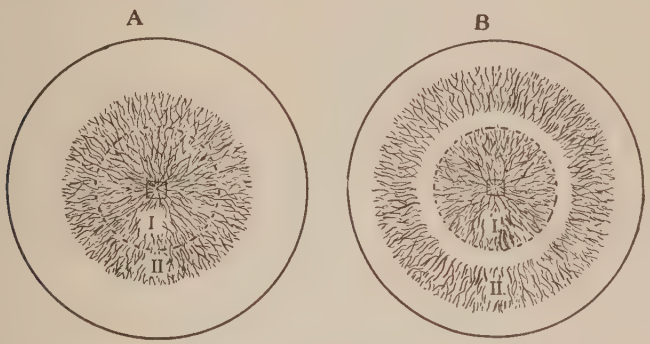
Result—The experimental results are shown in Table 6.

Table 6. Resistance of the fungus against bactericidallamp radition (Exp. 1).

Exposed time	Growth of the fungus	
	before exposed	after exposed*
0 minutes	13.19 cm^2	28.64 cm^2
40	13.19	38.69
50	13.04	40.01
60	13.19	40.12
90	13.32	42.67
120	13.22	40.01

* The area of fungual growth grown on 10cc Bouillon-agar medium at room temperature.

As shown in Table 6, all the exposed fungi survived and well grew, but the cultures after radiated showed a transparent zone about 2 mm in width around the growth before exposed (Textfig. 3).



Textfig. 3. Showing the growth of fungus (diagramatic).
A, Control; B, Exposed to bactericidal lamp-ray.
I, The growth of fungus at the begining of the experiment;
II, The growth after radiated, 24 hours cultured.

This phenomenon may result from the following fact: Although the fungi grown on the surface of the media were killed by the radiation yet the mycelia developed in the media remained survived and by their growth the discontinuity of the growth may have been arosed.

Morphologically, there was no difference in the fungal growth between the treated and non treated fungi. As it was conjectured from the above-mentioned experiment that the superficial fungi would be killed by the lamp-radiation for a

Table 7. Resistance of the fungus against bactericidal lamp radiation (Exp. 2).

Exposed time	Growth of the fungus	
0 minutes	19.01 cm^2 1)	100 % 2)
30	1.22	6.4
35	4.46	23.4
40	0	0
50	2.08	10.9
60	2.07	10.9
90	0	0
120	0	0

1) Growth after cultured at room temperature for 24 hours.
2) Relative percentage of growth determined as 100% of non-exposed fungal growth.

Notice: Initial growth of the fungus was almost same and showed 3.9~4.1 cm^2 in size.

certain temie so the second experiment was tried by the following method.

Sterilized cover glass was dipped in Bouillon broth, then it was placed by the side of the fungual-block which was transplanted on an agar-plate. The fungus was cultured at room temperature for 24 hours.

Thus cover glasses on which the fungi grew were obtained.

The fungi grown on the cover glass were exposed to the ramp-ray. The experimental results are shown in Table 7.

As shown in Table 7, a large part of the mycelia was killed by the radiation over 30 minutes and it is conjectured that the fungus would be killed completely by the radiation at least for 90 minutes.

At any rate, the fungus is well resistive to the bactericidal lamp-ray.

(5) Inoculative experiment.

Exp. 1—The experiments were tried in two ways, viz. infection by transplantation of mycelia and infection by zoospores.

Healthy eels about 100g in body weight obtained from Tokyo Fresh-Water Fish Association were used for the experiment. On the former experiment the fungus cultured on Bouillon-agar-plate at room temperature for 24 hours were inserted in a pocket made on the fish skin by cutting out the skin and then sewing up.

On the latter experiments, zoospores of the fungi and fungi block cultured on agar-plate were added in an aquarium (20 cm × 43 cm × 15 cm) in which fish had been kept. Before the experiments, the fish were washed well by boiled water. As the eels were kept to the experiments in ell-crate made of bamboo, so some slight abrasions were observed on their heads or tail portions. Therefore any pervious treatments were not given on the skin. The results are shown in Table 8.

Table 8. Results of inoculative experiment (Exp. 1).

Date	Nos. of fish	Inoculative method	Water temperature (°C)	Experimental result
19~25/V 1955	2	Transplantation of mycelia	18~19	All the fish were not infected and survived without any abnormality.
21~27/V 1955	3	Exposure to zoospores	13~15	Two fish were infected and one fish showed growth of fungus on head portion and another fish on tail portion. One fish was killed 25th May for examination and one fish died on 27th May. One remaining fish was not infected and survived without any abnormality.
21~27/V 1955	3	Transplantation of mycelia	13~15	New growth of fungus appeared on 23rd May in the transplanted part of one fish, the fish died on 27th May. Two remaining fish survived without any abnormality.

In the former experiments, one fish out of five was infected and the new growth of the fungi developed at the transplanted part two days after inoculation. The growth showed a tendency to spread and the fish died within six

days. Four remaining fish were not infected. In the latter experiments, two fish out of three were infected and the growth of fungi were seen on the head in one fish and on the tail of another fish two or three days after the starting of the experiments. One fish died in a period of six days.

Exp. 2—The experiment was carried out at the Yoshida Fish Cultural Laboratory of Tokyo University of Fisheries during June 11 to 17 in 1955.

In this experiment, very small ells measuring about 8 cm in body length under culturing at the laboratory and the fungus cultured on linseeds were used. Fifteen individuals of the eels were received in a glass vessel containing about 1.5 liters of water and three linseeds with well-grown fungi were put in it.

The vessil was placed in an aquarium with running water to keep the water temperature at about constant level. The water temperature showed 17–19°C during the experiment. The water in the vessel was exchanged according to the need. The results are shown in Table 9.

Table 9. Results of inoculative experiment (Exp. 2).

Date	Remark
11/VII, '55	The beginning of the experiment.
12	No abnormality found.
13	One fish was found infected.
14	Three more fish were infected.
15	Two fish died and two newly infected fish were found.
16	Two fish died and one newly infected fish was found.
17	One fish died.

As shown in Table 9, seven eels among fifteen were infected and five eels died during the experiment.

Unfortunately, the experiment was interrupted owing to unavoidable circumstances, and if it should have been continued, all the fish would have been infected.

All the dead fish had the growth of the fungus on almost entire surfaces of the bodies (Pl. I, Fig. 5) but the growth was merely superficial and affected parts were not apparently injured.

Under the microscope, the epidermis resulted in necrosis and collapsed.

Through the experiment, it was proved that the fingerling eels were weak to the fungus and spread of the fungus was very rapid.

6. Discussion

A disease of eels, so-called "Sure", occasionally appears in our eel-culture ponds and gives rise to not a little loss of eels. However, practically no attention has been paid to the disease. In the year of 1955 an explosive prevalence of the disease broke out and caused heavy loss of eels. Presumably, the very changeable water temperature in early spring of the year may be one of the causative factors.

As previously mentioned, the disease may be due to a species of water mold, *Saprolegnia parasitica* COKER.

Because it was limited to the period of comparatively low temperature and grew weaker rapidly with rise of water temperature. Further there was a slight evidence of disorder in viscera of affected fish.

Nevertheless some groups among diseased fish were infected with pathogenic bacteria belonging to *Pseudomonas*-group. Therefore it cannot be said the trouble is based uniquely on the fungus and the bacterial function cannot be neglected in some cases. However it is not doubtful that the fungus is a principal cause of the disease. In early spring, they may be liable to catch the infection, because they may be injured slightly by their habit of getting into mud during hibernation.

The mechanisms of the lethal function of the fungus to fish require further studies.

Discussions concerning to the fungus and control measures of the disease will be written after more studies are accomplished.

7. Summary

An investigation was made on a disease of eels which might have resulted from a kind of water mold, *Saprolegnia parasitica* COKER at Kawajiri district of Shizuoka Pref. in 1955.

The disease broke out in early March and continued to early May and grew weak rapidly when the water temperatures rose to about 18°C. During the epidemic, the water temperatures showed 14~21°C, on average 16°C.

The total mortality in a population of 81,442 Kilograms eels was 19.4 percent.

Diseased fish were lethargic, inappetent and exhibited heavy growth of fungi on the snout or the tail portion, sometimes on the trunk, but some groups among them showed the infection with a bacteria identical with *Pseudomonas punctata* f *sacrowiensis* SCHÄPERCLAUS or another type of *Pseudomonas* group. Hemorrhagic ulcers often developed at the parts affected with the fungi.

Experimental infection was accomplished by putting healthy eels in contaminated water with zoospores of the fungi or by transplanting the fungi in the skin. Fingerlings of eels were easily infected and died with the growth of the fungi.

Investigations concerning to the resistance of the fungus against some disinfectants and bactericidal lamp were made and results obtained were compiled in the Tables 3~7.

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PLATE I

Fig. 1. Showing the lesion of affected fish.

Fig. 2. Showing a histological figure of the affected skin. Sectioned in Celloidin, stained with Mayer's acid haemalaum and eosin. The upper is epidermal part. The epidermis completely disintegrated and covered with mass of fungi. Cu, Cutis. Also necrotic.

Fig. 3. Young mycelia of *Saprolegnia parasitica* COKER grown on eel-flesh-agar medium.

Fig. 4. Showing the culture of *S. parasitica* COKER on simple-agar-medium. Numerous zoospores are scattered. After 24 hours.

Fig. 5. Showing the growth of the fungi on experimentally infected eels.

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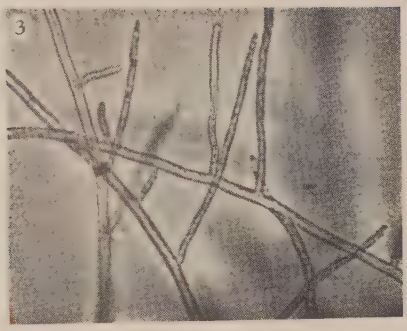
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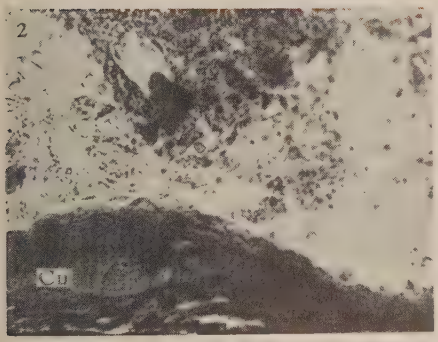
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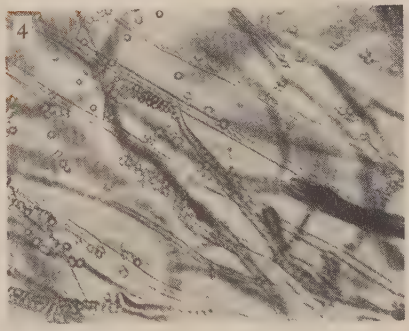
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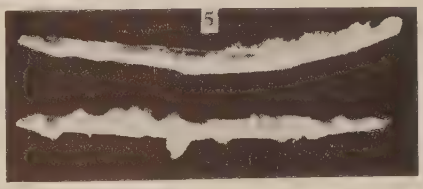
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Journal of the Tokyo University of Fisheries, Vol. 42, No. 1, 1956

AN EPIDEMIC DISEASE AFFECTING RAINBOW-TROUT IN JAPAN

Toshikazu Hoshina

(Received Feb. 8, 1956)

Recently, furunculosis like disease of rainbow-trout (*Salmo gairdnerii*) is prevalent at the fish culture ponds in various districts of Japan.

The loss of the rearing fish due to the disease is prodigious consequently it presents a significant problem in practical fish cultural management.

The author has been engaged in the study of the disease since 1953 and he could to examine the diseased fish obtained from the following places up to the present: (1) Gakunan Trout-Cultural Farm, Shizuoka Pref., April in 1953; Feb. and April in 1954. (2) Hakojima Trout Hatchery, Gunma Pref., May and Oct. in 1954. (3) Taisen Trout Hatchery, Tottori Pref., Nov. in 1954. (4) Mazuda Trout-Cultural Farm, Tottori Pref., Dec. in 1955.

The investigation revealed that the disease was caused by a kind of bacteria resembling to *Vibrio piscium* David, 1927. The writer here wish to make a previous report of the investigation. He wishes to express his hearty thanks to Messrs. Tetsutaro Ichijima, Director of the Gakunan Trout-Cultral Farm, Issei Kunemine, Director of Hakojima Trout Hatchery of Gunma Prefectural Fisheries Experimental Station, Misao Sôguri, Director of Tottori Pref. Fisheries Experimental Station, and Satoshi Suzuki, Director of Matsui Branch Office of Nippon Reizo Co., Ltd. for their aid in various ways, and to the Ministry of Education for the financial aid granted him.

Characteristics of the disease.—The severe prevalence of the disease was observed in late autumn to spring. The disease attacked the fingerling to adult fish but approximately one year old fish were mainly subjected to it. It was characterized by its highly infectious nature and by severe mortality rate. Diseased fish were characterized by the presence a boil like lesion resembling to those of furunculosis on the body or hemorrhagic lesions in the body musculatures (Pl. II, Figs. A,C). Some fish had hemorrhagic ulcer on the body or showed exophthalmos (Pl. II, Fig. B). The anus appeared congestive and dilative. In autopsied fish, the liver, intestine and peritoneum were congested generally; the spleen was enlarged and often disolved, the kidney was congested or necrotic. A few fish showed severe animia.

The smear preparations of blood, viscera and boil like lesions revealed without exception the presence of curved bacilli (Pl. II. Fig. D).

Attempts to isolate bacteria from blood, liver, spleen, kidney and boil like lesions yielded pure cultures of the microorganisms in the all cases.

To test for a pathogenity of the isolated bacilli, a suspension was prepared by adding ca. 4 mg of the cultured bacilli (on agar slope, at room temp. for 48 hours cultured) in 10 cc of 0.65% sterilized physiological salt solution and it was injected intramuscularly or intraperitoneally into a group consisting of 4 or 5

individuals of 12 to 20 cm healthy rainbow-trout (0.25 to 0.5 cc). All the fish died within 24 to 63 hours. They exhibited the disintegration and hemorrhage of musculature at the site of inoculation, hepatitis, peritonitis, interitis, splenic enlargement and dilation kidney etc. and cultures of liver, spleen, kidney and muscle on agar yielded colonies of same bacilli which were injected to the experimental fish.

The organism was also pathogenic for *Cyprinus carpio* and *Anguilla japonica*.

Description of the organism.—Morphology: Slightly curved, Gram-negative rod with somewhat pointed ends, measuring $1.3\sim2.7\mu\times0.5\sim0.9\mu$ (Pl. II, Fig. E), motile with polar flagellum non-sporing, non-chromogenic, non-encpsulated, and non-acid-fast. Agar slope: Smooth, glossy, gray whitish, semitransparent, moist. Agar plate: After 48 hours at 22°C the colonies circular, 0.5~2 mm in diameter, with a smooth moist convex surface, greyish-white in colour, translucent.

Bouillon and pepton water: Uniform turbidity, greyish-white pellicle and sediment.

Gelatin stab: Not liquefied.

Potato: Gleistining, smooth, moist, yellowish-brown. After several days medium becomes brownish.

Milk: After several days at 22°C coagulation occurs, later the coagulation dissolved.

Cultural characters: Aerob. Optimum temp. appears to be 15°~20°C. Growth occurs at 5°~30°C. No growth at 0° and 37°C. Optimum pH for growth 7.4; growth is possible at the pH range 6.3 to 9.7. Biochemical characteristics: H_2S and indol are both produced. Catalase reaction is positive. Methyl-red test and Voges-Proskauer test are both negative. Acid but no gas is produced in arabinose, glucose, mannose, galaktose, fructose, maltose, starch, mannit, and sorbit. No acid and no gas are produced in xylose, sorbose rhamnose, lactose sucrose, raffinose, inulin, dulcit, glycerin and slcin.

The organism reduces nitrate to nitrite but fails to reduce nitrite. Identity: The organism is identified with the genus *Vibrio*, the specific name will be given in future report.

Summary—The bacterial disease of rainbow-trout (*Salmo gairdnerii*) which happened in Shizuoka, Gunma and Tottori Pref. was studied. The causative agent was idetified with *Vibrio* sp. provisionally.

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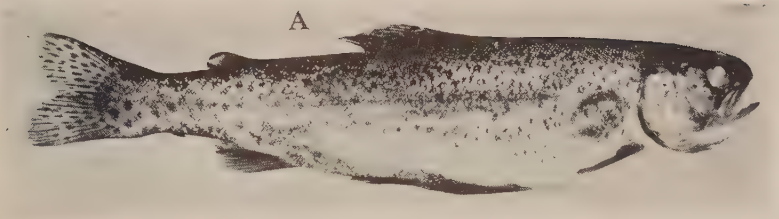
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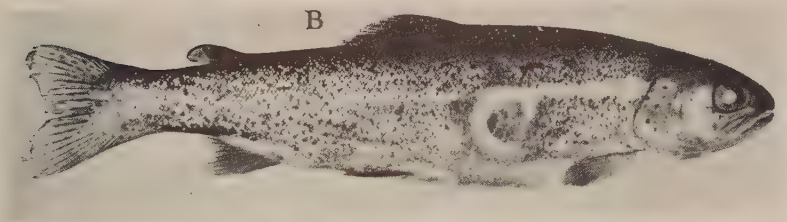
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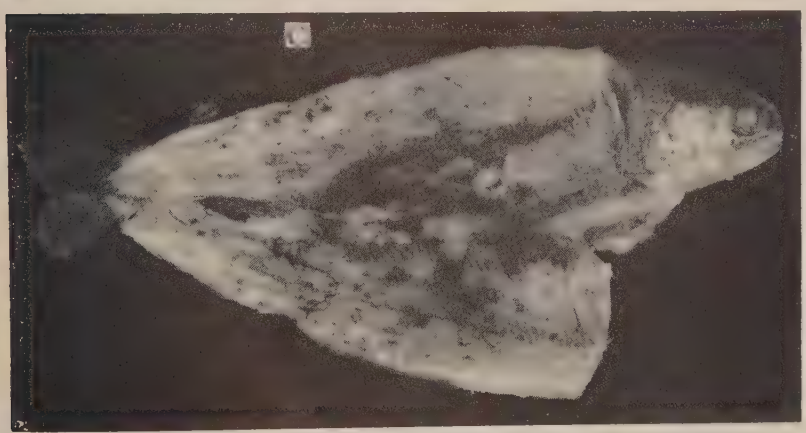
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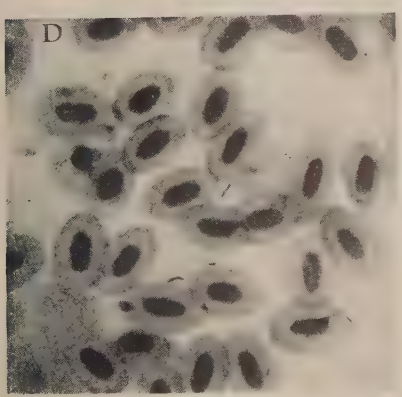
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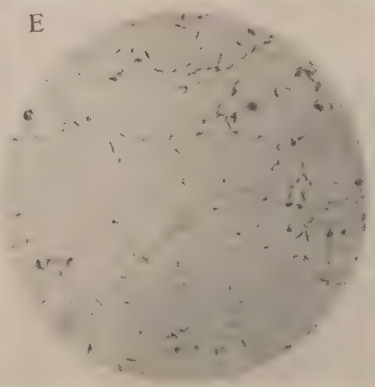
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PLATE II

Fig. A. The rainbow-trout showing the boil like lesion on the body upper the pectoral fin. Fig. B. Ulcerous lesion. Fig C. Numerous hemorrhagic lesions in the musculatures; viscera and peritoneum inflammed. Fig. D. Showing the pathogenic organism in the blood; smeared preparation of heart blood. May-Giemsa stain. Fig. E. Twenty-four hours culture of the organism on agar slope at 22°C, carbol-gentian violet stain.

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ON THE DIGESTIVE SYSTEM AND FEEDING HABITS OF YOUNG CHINESE CARPS COLLECTED IN THE RIVER TONE

Densaburo Inaba and Minoru Nomura

(Received Dec. 20, 1955)

Since as early as 1878, the fry of Chinese grass carp, *Ctenopharyngodon idellus* and silver carp, *Hypophthalmichthys molitrix* have been introduced to ur country from Shanghai. In the recent years, especially from 1941 to 1945, e fry of Chinese carps, nearly 4.7 millions in number, were transferred and stinated to 21 prefectures (Tange, 1949, Kuronuma '54). These fry were rear-l in fish pond and also stocked to natural lakes and rivers, i.e. Lake Biwa, iga Pref., Lake Togo, Tottori Pref., the River Tone and others. It was id that the Chinese carp grew normally wherever they transplanted, though ey never spawned. Till 1947, this had been held true in Japanese waters and d not propagated anywhere. Since 1947, however, the three species of Chinese rps, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix* and *Aristichthys ilis*, mostly the first two species mentioned above, have been seen frequently natural waters of a system of the River Tone, including Lake Kasumigaura, ake Kitaura, Lake Sotonasaka, and the River Watarase, etc. According to a cord of Ibaragi Prefectural Office, in the system of the River Tone, nearly ,000 fry were stocked during 1943 to 1945. Nowadays, it is clear to say that hinese carps naturally propagate in the river system, but it is difficult to ob-in sufficient numbers of adults of these carps, especially young ones even for e purpose of research (Tange, 1948, '49, Nakamura, '49, Inaba, '50, '54, Kuro-ma, '45).

Thus, new naturalized Chinese carps are propagated naturally among Japa-ese freshwater fishes, and it became necessary to investigate them concerning ith their food habits as well as ecological environments they dwell. Suyehiro 942) studied on the digestive system and feeding habits of many kinds of Japa-ese fishes but he was unable to obtain these carps, because they were not natur-ized at that time.

In China, Chen and Lin (1935), Lin (1935), Nie and Chiang (1954) and hers studied the life history of the grass carp, the structures of the gill-rakers d the food habits in large specimens of the silver carp, but there appeared no ntribution on the digestive system and feeding habits of the young of these prinid fishes. The present authors had a chance to collect a few young Chinese ass carps and silver carps and investigated their digestive system and feeding bits, and the results may be given in the following pages.

Authors express their hearty thanks to Mr. S. Takahashi, Chief of Fisheries ection of Ibaragi Prefecture, Mr. G. Suzuki, former head of Ibaragi Promotive isheries Experimental Station and Mr. M. Tange, former member of the Station, r their kind help to collect the materials.

Table 1. Data of collection and measurements of materials used in the present study.

Species	No.	Date	Locality	Body length	Body depth	Body weight	Intestine length	I. l. / B. l.	Gillrakers
				cm	cm	g	cm		
Ctenopharyngodon idellus	1	August, 1950	Akitsu, Lake Kitaura	10.9	2.8	28.0	23	2.1	—
	2	August, 1950	Akitsu, Lake Kitaura	13.4	3.1	46.2	27	2.0	19
	3	June, 29 1950	Kihara, Lake Kasumigaura	14.1	3.5	57.8	31	2.2	23
	4	——	Manchuria	32.4	7.3	562.0	63	1.9	23—24
Hypophthalmichthys molitrix	1	August, 1950	Shinshima, Lake Sotonasaka	5.2	1.45	2.6	15.5	3.0	1)
	2	August, 1950	Shinshima, Lake Sotonasaka	5.3	1.5	2.6	17.5	3.3	
	3	August, 1950	Shinshima, Lake Sotonasaka	5.4	1.5	3.1	18.4	3.4	
	4	August, 1950	Lake Sotonasaka	9.9	2.9	11.5	40.4	4.1	
	5	Aug. 22, 1950	Kamishima, Lake Kitaura	10.1	2.9	15.8	38.5	3.8	
	6	Sep. 18, 1950	Lake Sotonasaka	11.5	3.5	26.5	44.2	3.8	
	7	Sep. 18, 1950	Lake Sotonasaka	11.7	3.6	30.0	44.8	3.9	
	8	August, 1950	Kamishima, Laku Kitaura	36.7	10.9	875.2	252.2	7.8	

1) Not countable, see Fig. 6.

Materials and methods

The materials used here, except a large grass carp sent from Manchuria, are all natural propagated fishes in the system of the River Tone, Ibaragi Prefecture, and they were caught by a seine, a cast net and some kind of trap in 1950. Details on locality, date and the measurements of materials examined are shown in Table 1.

Observation were made macroscopically. The dissection of the fish followed the method adopted by Suyehiro (1942), in the order of mouth, jaws, tongue, pharyngeal teeth, gill-rakers, pharynx, oesophagus, intestine, liver, gall-bladder, pancreas, air-bladder, etc., and the configuration and construction of the digestive tract. The food habit was investigated microscopically using the contents taken from digestive organ.

Grass carp (*Ctenopharyngodon idellus*)

A. Digestive system. The upper jaw is a little longer than the lower and has thick lips. There are no teeth on jaws, vomer, palatines and tongue. The buccal cavity has many low longitudinal folds on the roof of mouth and a few transverse folds on the front part of it (Fig. 1). The labial fold runs along the upper lip. The tongue is not free from the floor of mouth cavity and consequently is not found at a glance, but the mucous membrane by which the tongue is covered rises a semitriangular form, and many fine transverse folds on the surface of the tongue. The gill-arch has no upper limb, with no bending. The gill-rakers

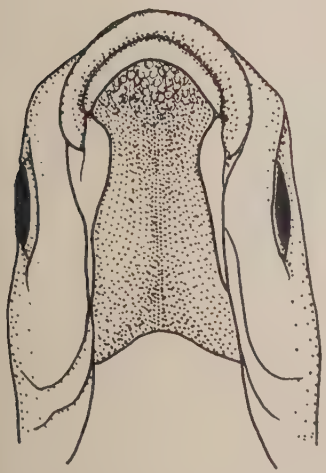

Fig. 1. Roof of mouth cavity of a grass carp 10.9cm long, ×2.

Fig. 2. Floor of mouth cavity of a grass carp 32.4cm long, showing arrangement of pharyngeal teeth, lp. Lower pharyngeal teeth, ×4/5.

are short, broad, grown thickly and 19 to 25 in number. Both upper and lower pharyngeal teeth well develope; the lower pharyngeal teeth are arranged in 2 rows (2.4–5.2), the upper pharyngeal teeth are presented by a oval plate with a few transversal grooves and 3 longitudinal grooves on the surface (Fig. 2).

Fig. 3. Digestive organs of a grass carp 13.4 cm long, ×1.6. R. Right side view. L. Left side view. k. kidney. gb. Gall-bladder. l. Liver. i. Intestine.

The peritoneal cavity is seen dark with melanin chromatophores scattered in the peritoneum. Stout and short oesophagus continues to the "Stomach-like part" (Suyehiro, 1942) with a strip which is narrow and compressed. The winding of the intestine is standardized as illustrated in Fig. 4. There is no true stomach, like other cyprinid fish; the stomach-like part has thick wall and corresponds to the stomach of other fish. This part curves in the shape of the letter of U at its ending and turns into a narrow intestine. The intestine is short and its length is about 2 times as long as the body length. MIGITA and HASHIMOTO (1949) have shown that the length of intestine, in their specimens (25.5–41.0 cm in body length), are 1.9–2.9 times as long as the body length, which are close to our examination. It is clear that the ratio of the length does not vary with the growth of fish as the digestive organ has taken adult form before grown such size in our limit of study.

The mucous membrane of oesophagus consists of high, longitudinal folds, but these

Fig. 4. Diagram showing intestinal winding of a grass carp 13.4 cm long, ×3.2.

folds turn into complicated, ramulous, high folds at the borders of oesophagus and stomach-like part. These complicated folds change into smooth, transverse folds gradually at the first 1/3 part of the stomach-like part. The wall of intestine has transverse folds, but it changes into longitudinal folds near the anus.

The liver is irregular form, spreads over the right side of mass of digestive tract but submerges into intestinal tract toward the back.

The pancreas is irregular form and situated on the left side of the omach-like part.

Gall-bladder is large, and consists of semitransparent, thin membrane with elanin chromatiphores scattering on the surface. It is located in the right side the stomach-like part. The bile-duct opens to the anterior end of the stomach-ke part.

The air-bladder is large and dumb-bell shaped, covering all over the dorsal de of the peritoneal cavity, and it consists of two chambers. The anterior hamber is larger than the posterior and is formed by opaque membrane, but osterior chamber by semitransparent membrane. The pneumatic duct starts om the lower part of the anterior end of the posterior chamber and communi-ates to the beginning of the stomach-like part.

B. Feeding habit. According to the results of rearing experiment of the y grass carp collected in the River Tone, we found that they took zooplankton ich as water flea before they grew about 3.5 cm in body length. After that they hanged their food, eating the fragments of bread and oligochaete worms (Tubi-cidae) fed by us. The omnivorous habit of this carp is remarkable seen in rear-g ponds as described by Chen and Lin (1935), Hora[1] (1953) and others. In le materials examined (Table 1), collected in the natural water, we did not find ny plankton, but found the detritus of water grass (*Sagittaria*, *Vallisneria*) as ell as some insects and diatoms which seemed to be eaten with the water grass. n our field observations in the River Tone, we have often experienced that the dult grass carp eat a large quantity of leaves of the water grass, the tender art of reed and other grasses only. From the facts mentioned above, it may e said that the grass carp is an omnivorous fish in the young stage, and shows tendency to change into the herbivorous with advance of growth in the natural aters.

Silver carp (*Hypophthalmichthys molitrix*)

A. Digestive system. The lower jaw is slightly longer than the upper. he lips are thin. The teeth are absent on the jaws, vomer, palatines and ton-ue. The roof of mouth cavity is the narrowest in its width at the position of e orbits. The anterior part of the narrowest portion is fan shaped and with folds. In the posterior part of it, there are three longitudinal folds which art from the position of the orbits and run along the both sides of the roof of outh cavity, respectively. Another three short longitudinal folds run in the iddle of the posterior part. There is no labial fold. The tongue is not free om the floor of mouth cavity. There are four gill-arches (Fig. 5).

The gill-rakers differ from those of other cyprinid fishes in the form and ructure as shown by Fang (1928) and Nie and Chiang (1954). They are membran-us and consist of numerous, elongated, filamentous rays and the reticulum with

1) Hora (1953, p. 37) described as follows; it is an omnivorous fish. Even though it ill take any kind of food that is available, it shows a definite preference for green grass nd vegetables than the food of animal origin.

structure of soft network (Fig. 6). The membranous gill-rakers are arranged two rows on each gill-arch; the two rowed rays run parallel with each other the middle line of gill arch and the reticulum co glutinates to the whole outer surfaces of two row rays (Figs. 5, 6).

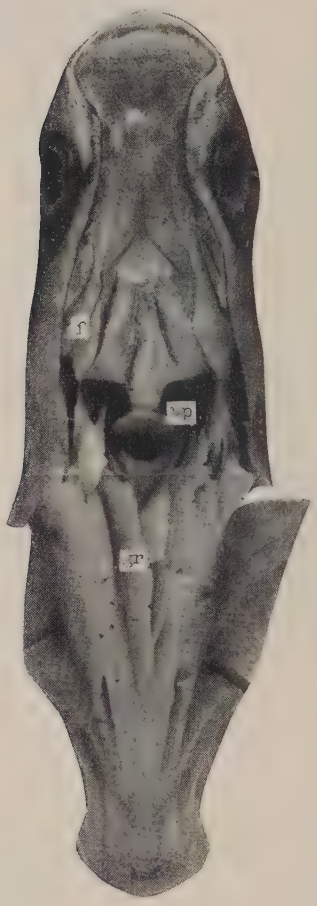

Fig. 5. Roof and floor of mouth cavity of a silver carp 10.1 cm long. up. Upper pharyngeal teeth. gr. Gillraker. f. Folds of roof of mouth cavity, ×2.3.

Both upper and lower pharyngeal teeth a developed. The lower pharyngeal teeth are a ranged in a row (4–4), and the grinding surfa is smooth and somewhat hollowed, an oval in shap The upper pharyngeal teeth are represented by shield-shaped plate with a longitudinal groove a a few transverse grooves on the surface.

Melanine chromatophore are scattered in t peritoneum. Stout and short oesophagus continu to the intestine with a strip which is narrow a compressed. The intestine succeeding oesophag is a little broader than the other parts. The r tio of the length of the intestine to the body leng increases with the growth, 3.0 to 3.4 times smaller ones, 5.2 to 5.4 cm in body length, 3.8 4.1 times in 9.9 to 11.7 cm in body length, 7 times in larger one, 36.7 cm in body length shown in Table 1. According to the study NIE and CHIANG (1954) in large specimens (30.7 41.0 cm in standard length) the ratio is 7.85 time which coincides with ours. The winding is ve complicated, and it varies with the growth. T fundmental winding, however, does not much var but increases only its convolution as shown Plate III.

The mucous membrane of the oesophag consists of longitudinal folds. The wall of t first 1/3 part of intestine has typical longitudin zigzag folds and succeeding it these folds gro into gradually low, somewhat irregular, longitud nal folds or fine papillae. There are longitudin folds near the anus.

The liver with the pancreas is the form the hepatopancreas. It is irregular, and situat on the mass of digestive tract. At the anterior portion it surrounds the intesti succeeding oesophagus; at the posterior it spreads over the right side of t mass of digestive tract and reaches to the left side.

The gall-bladder and air-bladder are very similar to those of the grass car

B. Feeding habits. The silver carp feeds mainly on plankton and is know as a plankton feeder (CHEN and LIN (1935), HORA (1953) and others). Judgi from the construction of gill-rakers, it seems the silver carp will take micr

organisms. Exactly as we expected main content of the digestive organ of the young silver carp of such size in Table 1, were of micro-organisms. They are

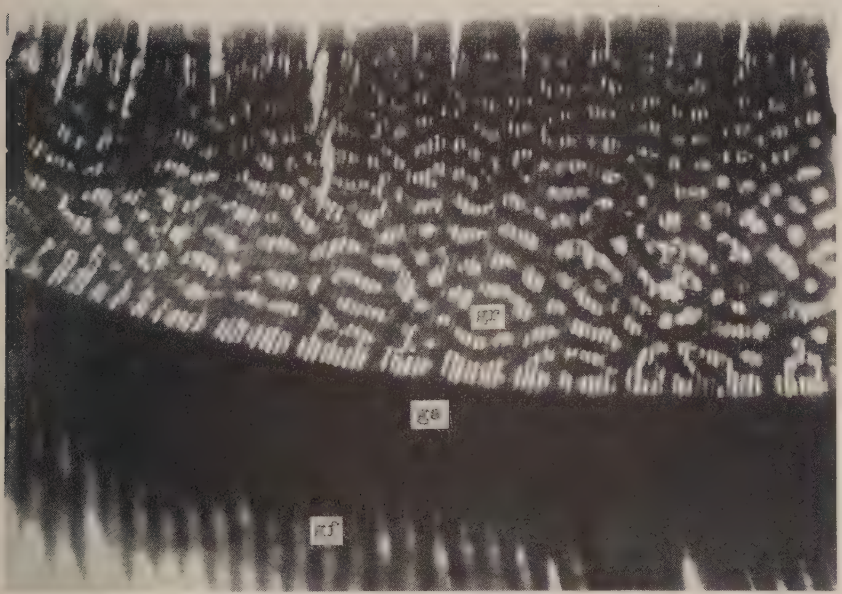

Fig. 6. Structure of gill of silver carp, ×19. gr. Gill-raker. ga. Gill-arch. gf. Gill-filament.

diatoms, algae and small particles of water grasses. Details are as follows:

Diatomaceae; *Melosira varians* (cc), *M. italica* (c), *Navicula* spp. (+) *Synedra ulna* (+), *Cymbella* spp. (r), *Epithemia* sp. (r), *Gomphonema* sp. (r), *Diatoma* spp. (+).

Chlorophyceae; *Scenedesmus* sp. (rr), *Ulothrix* sp. (rr), *Eudorina* sp. (rr).

Cyanophyceae; *Microcystis* sp. (+).

Detritus of some grasses (not identified).

According to Nie and Chiang (1954) large silver carp finding in the natural waters in China, feeds also chiefly phytoplankton though it often takes some zooplankton. We did not find any zooplankton. It seems to us that the young silver carp takes mainly phytoplankton and some periphytonic diatoms such as *Melosira varians*, *Navicula*, *Epithemia*, *Gomphonema*.

Summary

We examined the digestive system and feeding habits of young grass carp (10.9–14.1 cm in body length) and silver carp (5.2–11.7 cm in body length) collected in the system of the River Tone.

Gill-rakers, in the grass carp, are short, broad and thick, and 19 to 25 in number (Fig. 2). In the silver carp they are special membranous form, differ-

ing from other cyprinid fishes, which consist of long rays and a reticulum (Fig. 6) as shown by FANG (1928) and NIE and CHIANG (1954).

The winding of the intestine is complicated in these carps. But the grass carp is somewhat simpler than the silver carp as shown in Figs. 3, 4 and Plate III. The winding of the former does not vary with growth, but in the latter it varies with growth as shown in Plate III.

The ratio of the length of the intestine to the body length is nearly 2 times in the grass carp and does not change with its growth after a certain such size (Table 1). In the silver carp the ratio varies with the growth in such extent (Table III), that is, the smaller ones are 3 to 4 times as long as the body length, but the larger nearly 8 times (Table I).

Of the feeding habits it seems that the grass carp is an omnivorous till it grows about 3.5 cm in body length, after that, it shows a tendency to change into the herbivorous in the natural water. The young silver carp takes mainly phytoplankton and some periphytonic diatoms.

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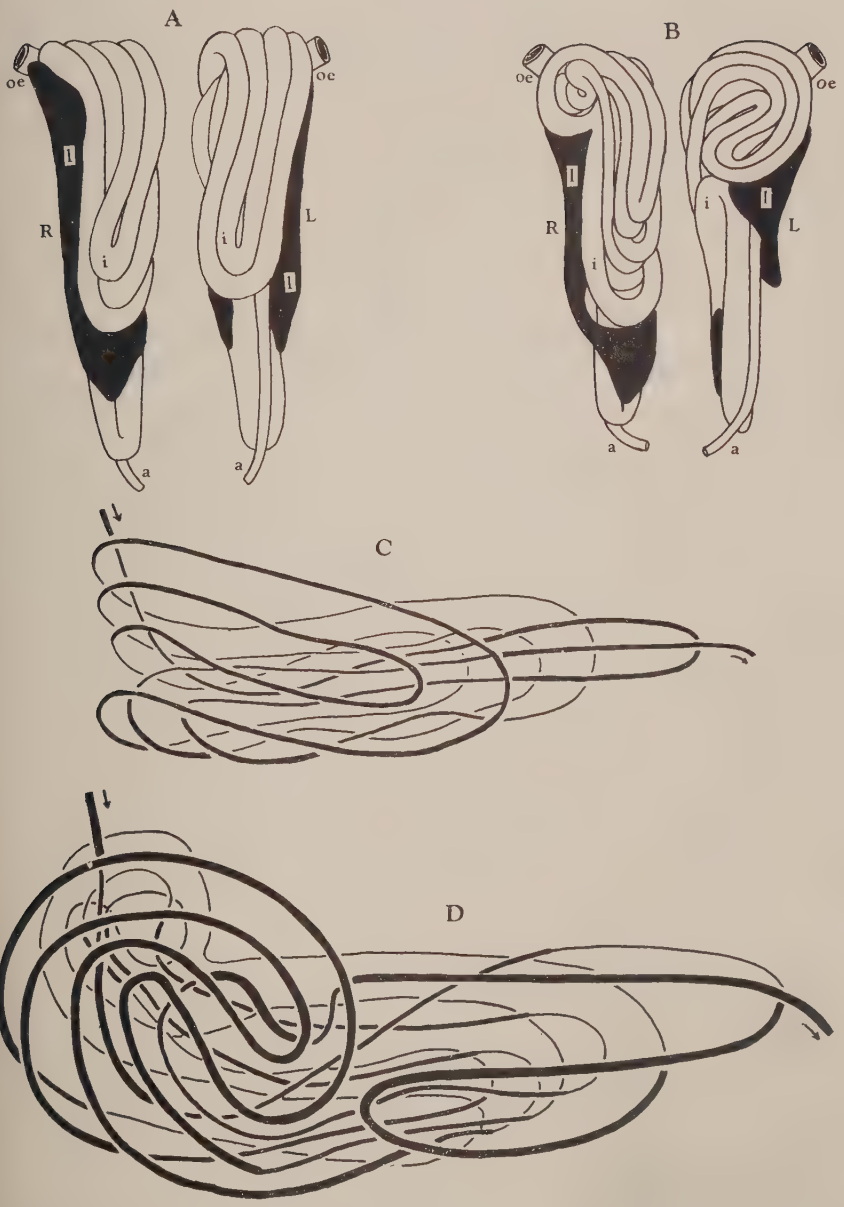

Pl. III. Digestive organs of silver carp (*H. molitrix*). A shows organs of a young 5.3 cm in body length, and B the same of a fish 10.1 cm long; L showing the organs seen from the left side and R from the right side, i indicating intestine, a. anus and l. liver; C shows the winding of the digestive tract of a young 5.3 cm in body length and D the same of a fish 10.1 cm long.

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DETERMINATION METHOD OF FRESHNESS OF FISH MUSCLE WITH TRIMETHYLAMINE

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Introduction

Many methods have been tried to test the freshness of fish muscle by etermining the quantity of the sole or a group of substance produced during poilage of fish muscle. The condition required to the substance for such purpose s that it does not exist in the fresh muscle or that its constant quantity exists nd increases regularly and clearly with the occurrence of putrefaction. From his point, it has been said that trimethylamine is more available than total olatile base, namely, in the fresh fish muscle, a considerable quantity of total olatile base exists and it is hard to say to be a constant quantity, while tri- ıethylamine content always is extremely so little that it can be practically isregarded, and with the occurrence of putrefaction by bacteria a large quantity f trimethylamine is suddenly produced[1]. Although it is considered that tri- ıethylamine is mainly produced from trimethylamine oxide by trimethylamine xide reductase of bacteria[2], the formation of trimethylamine during spoilage ıust be considered to vary according to the kind of fish, because the content f trimethylamine oxide is said remarkably to vary with the kind of fish[3]. The uthors will report their investigation on the practical value of the determination ıethod of the freshness of fish muscle by the quantity of trimethylamine about ome kinds of fish in Japan.

The authors wish to express their sincere gratitude to Mr. Michizo Suyama f their university for his kindness shown in various ways and his invaluable dvice throughout this study.

Experimental method

pH was determined in accordance with Hygienic Examination Guide III[4] P. 49–52) using glass electrode pH meter. Volatile basic nitrogen (VBN) was etermined by the boric acid method of Conway's micro diffusion analysis[5] (P. 5–97). Boric acid concentration used for this experiment was 1% and the tandard acid for titration was N/50 H_2SO_4. For the determination of trimethyla- ıine nitrogen (TMA-N), after triturating the 10 g fish muscle in a mortar, uthors added 20 cc 0.85% NaCl solution and agitated it, then after 10 minutes' xtraction, filtered it and took 1 cc filterate and determined TMA-N according to onway's mentioned method[6] (P. 176–181).

For the determination of trimethylamine oxide nitrogen (TMAO-N), authors, ı accordance with W. J. Dyer's method[7], put 5 g fish muscle in a mortar and fter triturating it, added 5 cc distilled water. Adding 10 cc 10% trichlor acetic

acid, they extracted it for about 2 hours and then filtered it, put the 5 cc filtrate in a test tube, added 1 cc 6N HCl and 0.5 g Devarda's alloy, treated it for 15 minutes in boiling water, reduced TMAO to TMA, made it up to 50 cc or 100 cc by adding distilled water. Then taking 1 cc of it, they determined TMA-N by the above-mentioned TMA-N determination method, and regarded numerical value as TMAO-N.

The freshness by the sensory test were shown as follows: rigor mortis (卌), freshness extremely good (卄), freshness good (＋), average freshness (+), freshness no good (±∓), incipient putridity (−), complete putrefaction (=).

Expeirmental procedure and results

1. *Comparison of three chemical determination methods on the freshness of fish muscle*

Authors put about 400 g of only the white muscle of each of 4 kinds of fresh fish (big-eyed tuna, mackerel, bonito, pig fish*) which were cut into about 5 mm cube, in a big Petri dish, kept it in the ice box at about 10°C for 6 days, and sampling everyday until putrefaction, authors determined pH, VBN and TMA-N about every sample. Besides, authors recorded the results of the sensory test. The experimental result is shown in the Table 1.

Table 1. A Comparison of some chemical tests of the quality of fish in the course of their putrefaction.

Sample	Days elapsed / Testing methods	0	1	2	3	4	5	6
Big-eyed Tuna	Sensory test	＋	+	−	=			
	pH	6.75	6.61	6.83	7.13	7.80	8.01	8.24
	VBN mg%	15.8	17.4	31.8	52.5	80.2	139.0	177.0
	TMA-N mg%	1.3	1.9	16.3	25.8	21.0	26.5	29.1
Mackerel	Sensory test	＋	+	+	∓	∓	−	=
	pH	5.92	6.00	5.92	6.05	6.47	6.94	7.44
	VBN mg%	16.0	17.4	23.1	26.7	28.3	37.3	66.4
	TMB-N mg%	0.4	0.4	1.6	5.9	17.8	19.2	8.7
Bonito	Sensory test	＋	+	+	+	∓	−	=
	pH	5.90	5.91	5.82	5.87	6.18	6.51	7.13
	VBN mg%	22.6	19.9	21.8	19.6	25.3	31.6	36.5
	TMA-N mg%	0.5	0.5	0.6	2.4	2.2	4.5	6.8
Pig Fish	Sensory test	卌	＋	＋	+	−	=	=
	pH	6.53	6.70	6.71	6.92	7.49	7.83	7.82
	VBN mg%	22.6	16.6	19.1	38.4	38.4	56.0	77.6
	TMA-N mg%	0.5	0.4	3.3	20.5	29.6	29.6	45.9

* Isaki in Japanese.

According to the Table 1, it is remarkable that although TMA-N is very ttle in all the 4 kinds of fish when they are fresh and increases with the pro- ress of their putrefaction, and in bonito, its increase is small while in pig fish : is extremely sudden and large. It can be said that with the kinds of fish TMA ɔrmation varies quantitatively.

Authors also compared the determination value by the 3 methods, and con- idered that TMA-N determination was the best method for detecting bacterial utrefaction of fish muscle, because of the fact that its numerical value when hey were fresh was comparatively definite while at the occurrence of putrefac- ion it changed suddenly.

2. Investigation of TMA formation during spoilage about many kinds of fish

(i) Besides sensory test and pH determination, authors determined TMA n the 15 kinds of such minced fish muscles as mentioned above when they were resh and after stored for several days in the ice box at 7-16°C. The samples f fish used for this experiment were got late in August, through a retail shop t Kurihama, Kanagawa Prefecture. The experimental results are shown in the Table 2.

Table 2. Trimethylamine content in 15 kinds of fish muscle before and after storage at 7-16°C. *Names given in brackets show Japanese names.

Species	Before Storage			After Storage			
	Sensory test	pH	TMA-N mg%	Days elapsed	Sensory test	pH	TMA-N mg%
Mackerel	##	5.83	0.0	4	−	6.88	30.3
Yellowfin Tuna	#	6.62	3.1	2	−	6.95	18.8
Pig Fish	##	6.59	0.2	3	−	7.87	28.0
Trigger Fish (Kawahagi)	##	6.33	0.0	3	±	7.11	31.1
Frigate Mackerel	#	5.66	0.2	3	+	6.10	4.5
Crimson Tai (Chidai)	##	6.50	0.9	2	±	6.70	14.1
Sea Robin	##	6.62	0.4	2	±	6.85	26.3
Horse Mackerel	###	6.35	0.3	2	±	7.43	36.8
Horse Mackerel (Small type)	##	6.51	0.6	2	±	6.96	17.5
Hair Tail (Tachiuo)	##	6.81	0.1	2	−	7.75	35.3
Barracuda	##	6.71	0.2	2	−	7.46	20.3
Gray Rock Cod (Mebaru)	###	7.16	0.2	2	−	7.65	21.2
Scorpion Fish (Oniokoze)	#	6.38	0.2	2	±	7.47	21.2
Wrasse	###	6.99	0.2	2	−	7.71	37.4
Surf Perch	###	7.04	0.2	2	−	7.67	14.6

According to the Table 2, any kind of fish contains exceedingly little TMA n its muscle when it is fresh, and after spoilage, produces a large quantity of .

(ii) In order to investigate in detail the condition of TMA formation on nore examples in the course of spoilage of fish muscle, authors performed the

same determination as mentioned above on ten more kinds of fish muscle, storing it in the ice box by the same way as above, sampling everyday. The samples of fish used for this experiment were got late in September, also at Kurihama. The results of the experiment are shown in the Table 3.

Table 3. Changes of trimethylamine content in 10 kinds of fish muscle during their spoillage. (Storage temp. 4–14°C)

Species	Days elapsed / Testing methods	0	1	2	3	4	5	6	7	8
Big-eyed Tuna	Sensory test	+		+	+	±	=			
	pH	6.55		6.66	6.51	6.83	6.70			
	TMA-N mg%	0.6		0.8	12.7	12.9	13.7			
Frigate Mackerel	Sensory test	+		+	+	+	±	±		−
	pH	5.30		5.72	5.66	5.79	5.80	5.68		5.78
	TMA-N mg%	0.2			0.2			0.3		0.4
Crimson Tai	Sensory test	∓		+	±	−				
	pH	6.41		6.55	6.78	7.15				
	TMA-N mg%	0.2		4.1	27.0	17.8				
Common Sea Bass (Suzuki)	Sensory test	+		+	±	=				
	pH	6.79		6.72	7.19	7.06				
	TMA-N mg%	0.6		4.3	20.9	32.1				
Mackerel	Sensory test	∓		+		+	+	±	∓	=
	pH			5.75		5.73	5.75	5.73	5.93	6.01
	TMA-N mg%	1.8		0.9		1.5	1.2	1.8	1.9	2.3
Saury (Sanma)	Sensory test	+		+	±	−	=			
	pH	5.72		6.25	6.18	6.42	6.90			
	TMA-N mg%	0.5		11.8	14.6	16.2	16.6			
Yellowfin Tuna	Sensory test	+	+	+	+	−	=			
	pH	5.99	6.03	6.13	6.10	6.41	6.68			
	TMA-N mg%	2.4	2.8			2.4	1.7			
Mackerel Scad (Muro-aji)	Sensory test	+	±	−	−	=				
	pH	6.13	6.04	5.98	5.95	5.88				
	TMA-N mg%	1.9	2.2	19.2	14.3	4.2				
Horse Mackerel	Sensory test	∓	+	+	±	±	±			
	pH	6.13	5.97	6.08	6.62	6.55	6.85			
	TMA-N mg%	0.4	1.3	21.2	27.7	32.9	43.1			
Barracuda	Sensory test	∓	+	+	−	−				
	pH	6.55	6.36	6.40	6.61	6.78				
	TMA-N mg%	0.5	1.5	10.4	19.9	25.2				

According to the Table 3, authors cannot recognize the evident increase of TMA-N during spoilage in frigate mackerel, mackerel and yellowfin tuna. Also in big-eyed tuna, crimson tai, common sea bass, saury, horse mackerel and barracuda, a large quantity of TMA is produced before authors functionally recognize the indication of putrefaction.

3. Determination of trimethylamine oxide content of common useful fish in Japan

Judging from all experimental results shown in the Tables 1–3, in so-called white-meat fish, without exception, TMA formation was remarkable during spoilage, while in the so-called red-meat fish, there were cases of little TMA formation. As the small content of TMAO might probably cause such little TMA formation in some red-meat fish, authors determined TMAO content of 11 kinds of common, useful fish in Japan. The samples of fish used for this experiment were got in Autumn at Kurihama, Kanagawa Prefecture. The experimental results are in the Table 4.

Table 4. Trimethylamine oxide content of common, useful fishes in Japan.

Species	No. of Sample	TMAO-N mg%								
Yellowfin Tuna	9	4.9	2.0	4.8	3.6	14.7	2.7	5.2	5.2	2.0
Big-eyed Tuna	3	3.3	13.4	16.2						
Mackerel	8	6.0	12.1	12.7	13.9	15.1	13.5	45.4	9.4	
Frigate Mackerel	3	0.8	3.9	2.9						
Saury	9	8.6	22.6	4.6	33.7	7.5	8.9	18.2	15.2	16.8
Sardine	3	9.4	7.1	7.9						
Horse Mackerel	3	56.4	44.0	56.8						
Round Flounder	3	30.0	46.0	50.4						
Surf Perch	4	47.2	38.6	37.6	37.6					
Common Sea Bass	6	55.4	52.3	50.6	49.3	54.6	61.2			

As shown in the Table 4, because TMAO-N content in most red-meat fish, such as yellowfin tuna, big-eyed tuna, mackerel, frigate mackerel, saury, sardine, is about 10 mg% and sometimes it is about 2–3 mg%, small quantity of TMA will reasonably be produced during spoilage in these kinds of fish, while in white-meat fish TMAO-N content is 35–60 mg% and exceptional numerical value is not reported. Horse mackerel shows the similarity to white-meat fish in the numerical value of TMAO content of fish muscle.

Study

From the fact that TMA content is exceedingly little in the fresh fish and with the growth of putrefaction bacteria, it is produced so suddenly that at the early stage of putrefaction, it will be more than about 20 times as much as that in the fresh fish, authors consider TMA estimation as a proper method for the

determination of the freshness of fish. However, in red-meat fish, common, useful fish in Japan, TMA formation during spoilage is irregular and only little in some cases. It is chiefly because of small TMAO content in these kinds of fish and the various TMAO content resulted from the difference of their individuals. As TMAO content is generally big in white-meat fish, TMA formation during spoilage is also remarkable, but as shown in the experimental results, it is hard to mark the limit of putrefaction by TMA-N mg%. However, in the completely fresh fish muscle, TMA-N content is generally under 1 mg%. If the content is over 10 mg%, it shows that bacterial number increases and might be a warning against incipient putridity or against its being just before incipient putridity. Although general formula cannot be made because of their few experiments, roughly speaking, we may be able to say that 10-40 mg% TMA-N formation of fish muscle during storage is significant to the detection of bacterial spoilage of fish. But the accurate quantitative value of TMA-N content of fish muscle between 10 and 40 mg% may not probably indicate the exact degree of its spoilage.

Judging from these accounts in the case of TMA-N determination for the purpose of detection of the bacterial spoilage of fish, the rough numerical value will be practically enough for the determination. This is widely different from the fact that VBN estimation requires comparatively accurate quantitative value.

Making use of titration at the half absorption in Conway's micro diffusion analysis to find the approximate numbers of TMA content, can reduce the time of its determination. For an example of practical TMA-N determination of fish muscle, authors added 20 cc of 0.85% NaCl solution to 10 g minced fish muscle with a little sea sand, filtered it after extracting for 1 minute or 2, put its 0.5 cc filtrate into the outer chamber of Conway's unit after 1% boric acid solution with indicator is filled in its inner chamber.

After adding 0.5 cc formaldehyde solution to the outer chamber and mixing it, authors smeared vaselin on the lid and placed it on the unit, then opening the lid a little, poured 1 cc saturated K_2CO_3 solution into the outer chamber, put on the lid again, rotated the unit, and mixed it. After keeping for only ten minutes in the thermostat at 35°C, authors opened the lid and immediately titrated amine absorbed by boric acid with N/100 H_2SO_4 solution. As this titration value will show 40-50% of the numerical value at its complete absorption, authors can find out approximate TMA content from that.

Summary

There are remarkably differences in quantity according to the kind of fish in TMA formation in the fish muscle during spoilage. Authors have many examples of red-meat fish in which TMAO content is generally little and TMA produced during spoilage is also little and irregular, TMAO content is comparatively large in white-meat fish, and TMA production is also sudden and much.

Throughout all kinds of fish, TMA-N content in the fresh muscle is generally under 1 mg%.

It may be said in the case of white-meat fish that we can apply the determination of TMA content to the detection of bacterial spoilage of fish muscle.

n the case of white-meat fish, however, authors cannot consider that TMA-N ng% can show the degree of putrefaction, because the speed of TMA production raries according to the individual case. The fish is quite fresh when TMA-N content is under 1 mg% and when the content is above 10 mg% its bacterial number increases so much that the fish is in the condition of putrefaction or the condition akin to putrefaction.

It must be specially mentioned that because the author's experiments are on the examples of putrefaction limited in kinds and conditions further comprehensive and practical investigation is necessary for its practical application.

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HISTIDINE MEDIUM TEST FOR DISTINGUISHING METHOD OF FECAL COLI TYPE BACTERIA

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Introduction

Coliform organisms have been usually employed for the detection of fecal contamination in foods, water, soil and other materials. But the group contains not only coli type bacteria which inhabit the intestinal canal of men and animals, but also aerogenes and intermediate type bacteria generally distributed in soil, water, vegetables and grains.

In order to distinguish coli type bacteria from the other nonfecal strains methylred test, Voges-Proskauer test, citrate test and indole test are usually used. Gas ratio of CO_2:H_2 fermented, fermentation of cellobiose, inosite, glycerine and other carbohydrates, utilization of uric acid and Eijkman method are occasionally adopted for this purpose.

I prepared synthetic media which contained sole amino acid as the source of carbon and nitrogen, inoculated all types of coliform bacteria in the media and after incubation, investigated the occurrence of their growth.

In most cases of many kinds of amino acid, the growth of bacteria in such media was simultaneous or not through every type of coliform group.

In the synthetic medium contained histidine as the sole nutrient substance, the growth of fecal coli type bacteria did not occur and that of other nonfecal bacteria did. This synthetic medium containing histidine is presumably available for the distinguishing method of coli type bacteria from the other type in the coliform group.

I wish to express my sincere gratitude to Dr. Yutaka Sekine, Professor of our university, for his kind direction and advice throughout this study. I am indebted to Messrs. Yuyo Kuwana and Tasuku Furusawa for their support in many parts of the experiments in this study.

I also offer my hearty thanks to Professor Dr. K. Aiso and Mr. S. Nakajima of the Chiba University, and Mr. K. Matsumoto of the Tokyo Metropolitan Laboratories for Medical Science for their distribution of the bacterial strains.

Experiment

Investigation of many kinds of amino acids as the sole nutrient substance in the media

I prepared some synthetic media which contained a kind of amino acid as the sole nutrient substance. 12 kinds of amino acids were used on these media and each medium contained 0.1 g nitrogen of amino acid, 5.0 g NaCl, 2.0 g K_2HPO_4 and 0.3 g $MgSO_4 \cdot 7H_2O$ as mineral salts per 1 liter. After each medium was

modified to pH 7.0 with Na_2CO_3 solution, every 5 cc was taken in test tubes, and sterilized for 3 days by the usual method. Besides the above-mentioned media histidine medium which contained 0.5% glucose as the carbon source was specially prepared.

19 strains of coliform bacteria composed of every type in the Imvic system[1] were selected.

After every strain was cultured for 24 hours on the nutrient agar slant at 37°C, one loopful of it was suspended into 10 cc of phisiological salt solution, and one loopful of the suspension was inoculated in the test tubes which contained the above-mentioned media. After incubated for 4 days at 35°C the growth of bacteria was investigated. The result of the experiment is shown in the Table 1.

Table 1. Growth of coliform bacteria in the various amino acid media.

No. of Strain	Type of Strain	L-Glutamic acid	L-Aspartic acid	L-Proline	DL-Alanine	L-Histidine $HCl \cdot 2H_2O$	L-Histidine $HCl \cdot 2H_2O$ and glucose	Glycine	DL-Methionine	DL-Ornithine	L-Leucine	DL-Arginine	L-Lysine	DL-Tryptophane
1	Coli I	##	##	##	##	−	##	−	−	−	−	−	−	+
2	Coli I	##	##	##	##	−	##	−	−	−	−	−	−	+
3	Coli I	+	##	##	##	−	##	−	−	−	−	−	−	+
4	Coli II	##	##	##	##	##	+	−	−	−	−	−	−	+
5	Coli II	##	##	##	##	##	##	−	−	−	−	−	−	−
6	Coli II	##	##	##	##	##	+	−	−	−	−	−	−	−
7	Coli II	##	##	##	##	∓	+	−	−	−	−	−	−	−
8	Intermediate I	##	##	##	##	+	+	−	−	−	−	−	−	−
9	Intermediate I	##	##	##	##	##	+	−	−	−	−	−	−	−
10	Intermediate II	##	##	##	##	##	+	−	−	−	−	−	−	±
11	Intermediate II	∓	+	##	+	##	##	−	−	−	−	−	−	+
12	Intermediate II	##	##	##	##	##	##	−	−	−	−	−	−	+
13	Intermediate II	+	+	+	+	∓	∓	−	−	−	−	−	−	+
14	Aerogenes I	##	##	##	##	##	###	−	−	±	−	+	−	+
15	Aerogenes I	##	##	##	##	##	##	−	−	−	−	−	−	+
16	Aerogenes I	##	##	##	##	##	##	−	−	−	−	−	−	−
17	Aerogenes II	##	##	##	##	##	###	−	−	−	−	−	−	+
18	Aerogenes II	##	##	###	##	##	###	−	−	−	−	−	−	±
19	Aerogenes II	##	+	##	##	+	##	−	−	−	−	−	−	+

As the Table 1 shows, when glutamic acid, aspartic acid, proline and alanine were used respectively, all strains could grow, on the contrary, glycine, methionine, ornithine, leucine, arginine and lysine showed no growth of all strains.

Especially in the case of histidine containing medium, the strains of coli type 1 showed no growth and most strains of the other five types could grow

except rare strains. In the case of tryptphane medium the relation between growth of bacteria and their types was irregular.

As mentioned above, among many kinds of amino acid media, only histidine medium was found interesting for distinguishment of coli type bacteria in the coliform group.

Further investigation of histidine medium test for distinguishing method of fecal coli type bacteria.

From foods, faces, soil and vegetables, I isolated many strains of coliform group, applied Imvic test and histidine medium test to these strains and investigated the results of both tests. The medium we used contained 0.05% of L-Histidine·HCl·$2H_2O$ and the above-mentioned mineral salts.

The strains described in the Table 2 were given from Institute for Putrefaction Research, Chiba University and Tokyo Metropolitan Laboratories for Medical Science and all other strains described in the Tables 3–5 were collected at our laboratory.

Table 2. Imvic test and histidine test of coliform group bacteria isolated from foods.

No. of Strain	Indol	MR	VP	Citrate	Histidine	Type
20	+	+	−	−	−	Coli I
4	−	+	−	−	+	Coli II
1	−	+	−	−	∓	Coli II
9	−	+	−	+	+	Intermediate I
5	+	+	−	+	+	Intermediate II
1	+	+	−	+	∓	Intermediate II
17	−	−	+	+	+	Aerogenes I
4	+	−	+	+	+	Aerogenes II

Table 3. Imvic test and histidine test of coliform group bacteria isolated from feces.

No. of Strain	Indol	MR	VP	Citrate	Histidine	Type
88	+	+	−	−	−	Coli I
2	−	+	−	+	+	Intermediate I
4	+	+	−	+	+	Intermediate II
1	+	+	−	+	∓	Intermediate II
2	+	+	−	+	−	Intermediate II
1	+	+	−	±	−	Intermediate II
6	−	−	+	+	+	Aerogenes I
1	−	∓	+	+	+	Aerogenes I
2	+	−	+	+	+	Aerogenes II
2	+	±	+	+	+	Other type
1	+	+	±	+	+	Other type
6	−	±	+	+	+	Other type

Table 4. Imvic test and histidine test of coliform bacteria isolated from soil.

No. of Strains	Position of soils	Indol	MR	VP	Citrate	Histidine	Type
4	F_1	+	+	−	−	−	Coli I
4	H_1	+	+	−	−	−	Coli I
4	H_2	+	+	−	−	−	Coli I
4	H_3	+	+	−	−	−	Coli I
3	H_4	+	+	−	−	−	Coli I
1	H_4	+	+	−	∓	∓	Coli I
2	H_5	+	+	−	−	−	Coli I
3	H_6	+	+	−	−	−	Coli I
2	F_2	+	+	−	−	−	Coli I
1	F_3	+	+	−	−	−	Coli I
5	H_7	+	+	−	−	−	Coli I
1	H_8	+	+	−	−	−	Coli I
1	H_5	+	+	−	±	±	Intermediate II
1	H_6	+	+	−	+	+	Intermediate II
1	H_6	+	+	−	±	∓	Intermediate II
1	F_2	+	+	−	+	+	Intermediate II
1	F_3	+	+	−	±	+	Intermediate II
2	F_3	+	+	−	±	±	Intermediate II
2	H_8	+	+	±	+	+	Other type

Table 5. Imvic test and histidine test of coliform bacteria isolated from vegetables.

No. of Strain	Vegetable	Indol	MR	VP	Citrate	Histidine	Type
1	Cabbage	−	−	+	+	+	Aerogenes I
1	Cherry	−	−	+	+	+	Aerogenes I
1	Cherry	−	+	−	+	+	Intermediate I
3	Leek	−	−	+	+	+	Aerogenes I
1	Carrot	+	±	+	+	+	Other type
1	Scallion	−	−	+	+	+	Aerogenes I
1	Scallion	−	+	+	+	+	Other type
5	Egg-plant	−	−	+	+	+	Aerogenes I
2	Egg-plant	−	±	+	+	+	Other type
10	Field-pea	+	+	+	+	+	Other type
2	Field-pea	−	−	+	+	+	Aerogenes I
2	Field-pea	+	−	+	+	+	Aerogenes II
3	Broad bean	−	−	+	+	+	Aerogenes I
5	Tomato	−	−	+	+	+	Aerogenes I
1	Tomato	−	±	+	+	+	Other type
5	Tomato	−	+	+	+	+	Other type

116 strains described in the Table 3 were isolated from Endo plate agar culture of 29 specimens of human feces. The strains described in the Table 4 were isolated from 11 soil specimens which were collected on hills to the west of the Tokyo University of Fisheries at Kurihama, Kanagawa Prefecture and in the garden of the University. In this Table F and H show flat ground and hilly region respectively.

Experimental results showed in the Table 2–5 proved that all strains of coli type I, intermediate type I, aerogenes type I and II behaved equally without any exceptional case in both tests of citrate and histidine. Most strains of coli type II proved negative by the citrate test, on the contrary, was proved to be positive by the histidine test. In the case of intermediate type II most strains was proved to be positive at both tests, but the rare strains showed no growth in the histidine media.

After all, coli type I which is a typical fecal type in the coliform group was proved to be negative by the histidine test and the other types which contained chiefly nonfecal strains was proved to be positive except rare cases in intermediate type II.

Conclusion

Although a great many studies ever performed already, still the classification of coliform group bacteria by means of biological character is a difficult problem. Practically I can consider that this group contains coli group, aerogenes group and intermediate group. In the Imvic system coliform group is classified into six types. We generally regard that among these types, fecal coli bacteria show negative under the histidine test, but the other nonfecal types positive under the histidine test. For the practical use of histidine test for the detection of fecal coli bacteria in the coliform group is required further more investigation.

Summary

I prepared synthetic media which contained the sole kind of amino acid for nutrient material, in which media used 12 kinds of amino acid.

Many strains of every type in the coliform group were inoculated in these media and the occurrence of growth was investigated. In these experiments I investigated that specially the strains of coli type I did not grow in histidine medium while the other type grew well in this medium.

Then I collected many strains of coliform group from foods, soil, feces and vegetables, and with these strains performed Imvic test and the histidine test comparatively.

Among the all types of coliform group, all strains of coli type I and rare cases of coli type II and intermediate type II showed under the histidine test negative and on the contrary, all strains of the other type positive.

I suggested the possibility to distinguish fecal coli type bacteria from th other coliform group bacteria by the method of histidine medium test.

Reference

1) PARR. L. W. 1939. Coliform bacteria. Bact. Rev., 3, 1–48.

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POLAROGRAPHIC STUDIES ON THE RANCID OIL

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Introduction

Like other organic materials, fats are subject to deterioration, and the chief form of degradation in the case of fats is referred to as rancidity. The reactions involved in producing rancidity may be hydrolytic or oxidative in character, and may originate from enzymic activity or from spontaneous atmospheric oxidation.

The autoxidation of fats, oils, and fatty acids has long been recognized at two rather well-defined stages. The first stage constitutes a so-called inductive period, during which peroxides are formed which probably act as both catalysts and reactants at the later stage of the oxidation.

Organoleptic rancidity is generally first detectable at the end of this induction period. The later stage of autoxidation is accompanied by oxidative changes leading to the formation of hydroxy acids, hydroxy-keto acids, unsaturated hydroxy acids, lactones, and various condensation products, and finally by cleavage to aldehydes, aldehydo-acids, and mono- and dicarboxylic acids.

Many of the tests for indicating rancidity, such as the Kreis test[1] employing phloroglucinol in the presence of hydrochloric acid, are specific for the presence of aldehydes. It has been claimed that the color developed by this reagent is due to the presence of an epihydrin aldehyde.

Most of the tests for aldehydes, as well as for peroxides, can likewise serve as a test for rancidity. The reduction of methylen blue[2] and the Schiff's test have been proposed for this purpose.

As a test for aldehydes, Issoglio[3] used as a test the amount of potassium permanganate that was required to oxidize the water-soluble aldehydes extracted from the fat by shaking with distilled water.

Oxygen absorption has also been used to estimate rancidity, but it is of more use in the determination of incipent rancidity or stability of fats. Paper-chromatographic or spectrophotometric analysis of 2, 4-dinitrophenylhydrazone of carbonyl compound involved in oxidized fat and oil has also been available for the investigation of the rancidity of fat and oil.

Recent reviews on the subject of rancidity include the comprehensive report of Lea[4] and those of Beadle[5], Mattill[6], Olcott[7], Vibrans[8], Olcott and Mattill[9], Lundberg[10], Riemenschneider and Ault[11], and Quakenbush[12].

Lewis and Quackenbush[13] outlined a polarographic procedure that permitted them to study of water-insoluble compound occurring in fats, esters and hydrocarbones.

Willits et al[14),15] extended the application of the polarographic method to the study of fat autoxidation products by determining the polarographic waves

of 41 pure oxygen-containing compounds.

The compounds contained functional groups which are known or suspected to occur in autoxidized fats.

Nogami et al[16], observed a reduction wave due to the double bond of oleic acid, besides two distinct waves and a maximum wave due to unstable peroxides that formed in the initial stage of autoxidation.

Although many above-mentioned methods are available for the investigation of the rancidity of fats and oils, little attention has been given to its investigation.

This paper describes a trial of convenient method for the qualitative and quantitative determination of the deterioration products of water soluble compounds in rancid fats and oils.

Experimental

In the present paper the following polarographic studies are involved.

1. Preliminary experiment, the observation of the difference of polarograms between fresh and oxidized fish oils.
2. Polarographic investigation of fresh fish oils.
3. Polarographic investigation of oxidized fish oils.
4. Polarographic investigation of oxidized unsaturated fatty acids.
5. Polarographic investigation of water soluble compounds separated from oxidized fish oil by steam distillation.

I. Preliminary experiment.

The difference of polarographic waves among fresh and oxidized oils indicate to the possible usefulness of the polarographic method for the study of autoxidation products.

Therefore the authors made a preliminary investigation on the fresh and oxidized fish oils in 0.1N lithium hydroxide solution.

Procedure and method

(1) Sample. Fresh and oxidized sardine oils were used for this experiment. Freshly prepared sardine oil had acid value 5.36, iodine number 180.5, and peroxide value 0.12; and the oxidized sardine oil (oxidized by the aeration at 50°C for seven days) had acid value 15.42, iodine number 156.5, and peroxide value, 115.2.

(2) Apparatus. The polarograph used in this work was manufactured by the Yanagimoto Company, Kyōto, having a sensitivity of galvanometer 1.04×10^{-9} amp/mm/m. All half-wave potentials were corrected for IR drop and measured against a saturated calomel electrode.

(3) Solution. A 0.1N LiOH was used for the supporting electrolyte. Two ml. of sardine oil was added with 50 ml. of 0.1N LiOH solution and shaked vigourously for about five minutes, and layer part of the 0.1N LiOH solution was separated. In this experiment, dissolved oxygen was not removed by the bubbling of hydrogen or other inert gases.

Results. As shown in Fig. 1, fresh sardine oil showed two polarographic waves in 0.1N LiOH solution having the half-wave potentials of −0.4V and −1.2V vs S.C.E..

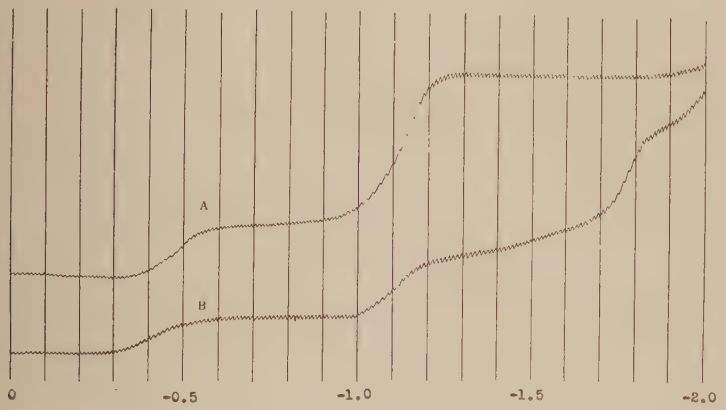

Fig. 1. Polarograms of fresh and oxidized sardine oils. A, fresh sardine oil; B, oxidized sardine oil. The supporting electrolyte was 0.1N LiOH.

In the case of the oxidized sardine oil, another polarographic wave was obtained. The half-wave potential of this wave was −1.9V vs S.C.E.. It seemed that the reduction wave was responsible for the oxidation products of sardine oil, for the fresh sardine oil did not show the polarographic wave.

The investigation of the reduction wave is available for the study of oil oxidation, and it is expected to get a clue to determination of the rancidity of oil deterioration.

Therefore the authors tried a detail examination of these polarographic waves. The increase and decrease of the reduction waves during the oxidation of fish oil, and a trial of decision for them are described in the later chapter.

II. Polarographic behaviors of fresh fish oils.

It is necessary to investigate the polarographic behavior of the oxidized fish oil, to know polarographic behavior of fresh fish oil. Therefore in this chapter the authors describe the polarographic behavior of fresh fish oil. For sample, freshly prepared sardine oil, whale oil, calamary oil, tunny fish oil, lard, beef tallow, and soya bean oil were used.

(1) Polarograms of fresh fish oils.

The polarograph used and the procedure of electrolytic solution were described the same as in the preliminary experiment. Two ml. of each fused fat and oil was added with 50 ml. of 0.1N LiOH solution and shaked vigourously for about five minutes, and layer part of the 0.1N LiOH solution was separated and used for the polarographic method.

As shown in Figs. 2 and 3, the same polarographic waves as described in the preliminary experiment were obtained in all samples. These waves had half-wave potentials of nearly −0.4V and −1.2V vs S.C.E..

(2) Change of wave heights by the expellation of dissolved oxygen. In the ex

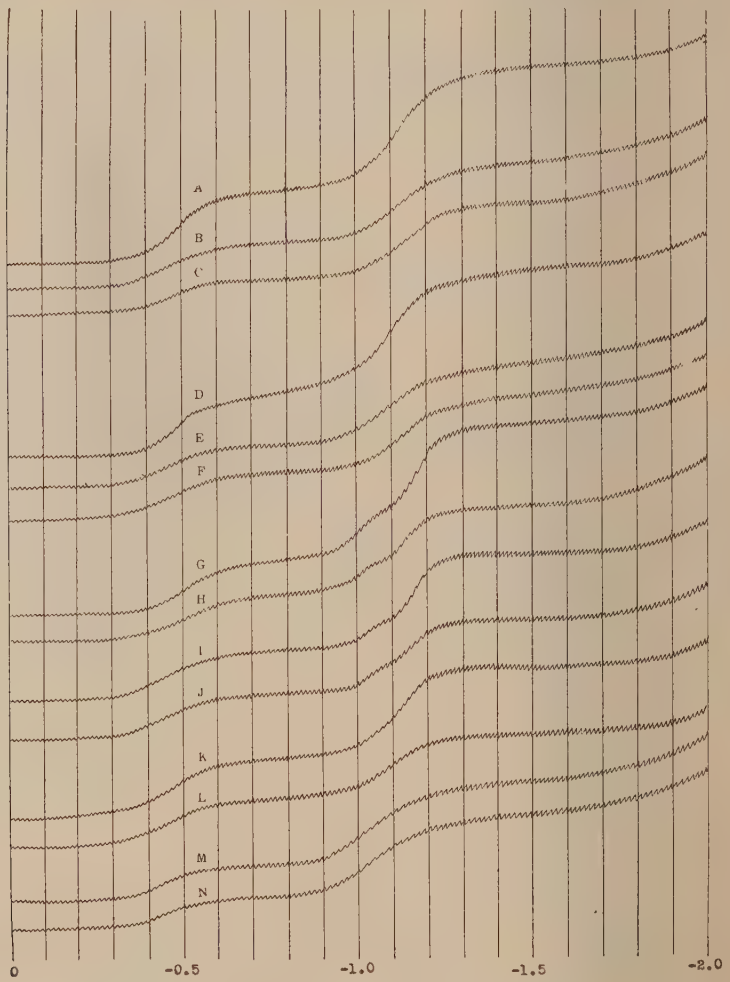

Fig. 2. Polarograms of fresh fats and oils in 0.1N LiOH. A, B and C were of sardine oil; D, E and F, tunny fish oil; G and H, whale oil; I and J, calamary oil; K and L, lard; M and N, beef tallow. A, D, G, I K and M did not removed the dissolved oxygen. B, E, H, J, L and N were expelled the dissolved oxygen by bubbling through H_2 gas for one hour, from the electrolytic solutions; and C and F, bubbling through H_2 gas for two hours.

periment of the former section, the observations of polarograms were carried out without removing the dissolved oxygen in elecorolytic solutions.

In this section, hydrogen, nitrogen or carbondioxide was used to expel the involved oxygen by bubbling through the solution for one and two hours.

The polarographic waves decreased in heights as the hours of gas bubbling

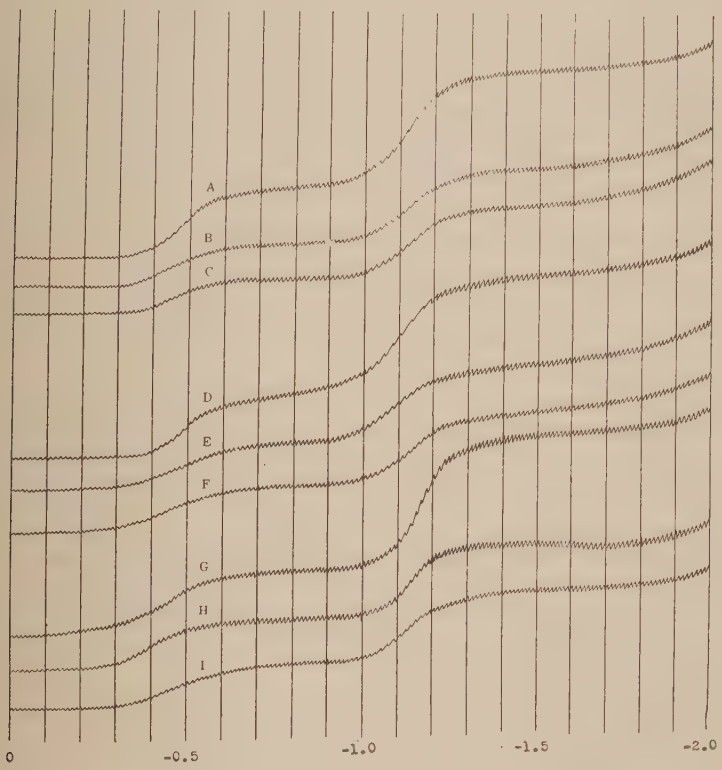

Fig. 3. Polarograms of fresh fats and oils in 0.1N LiOH. A, B and C was sardine oil; D, E and F, tunny fish oil; and G, H and I, soya bean oil. A, D and G did not removed the dissolved oxygen. B, E and H were expelled the dissolved oxygen by bubbling through N_2 gas for one hour from the electrolytic solutions; and C, F and I, bubbling through CO_2 gas for one hour.

increased. The decreasing delay of wave height by the bubbling hydrogen was the same as bubbling of nitrogen and carbondioxide.

The inclination of the decreasing heights by the bubbling of hydrogen, nitrogen and carbondioxide was represented in Figs. 2 and 3, and quantitative observation of decreasing was abridged, because the rate of decrease of wave height was not proportional to the bubbling hours of hydrogen, nitrogen or carbondioxide.

Nogami et al[16]. expected to know the degree of fat deterioration by the

polarographic waves which were reduced in methanol-NaCl solution, but the wave height was not proportional to the chemical peroxide value, because the wave height was not stable for the expellation of involved oxygen. They also indicated that the observed two waves were due to double bond and unstable peroxides formed at the initial stage of autoxidation of fat.

Gosman[17] suggested the applicability of the polarographic method to the determination of aldehydes and peroxides presence in ether. In this report the polarograms of KOH solution shaked with ether which was involved in aldehyde or peroxide, showed a polarographic wave reduced by the aldehyde or peroxide.

It seems that the investigation of Nogami et al was common to the authors investigation on the point that the polarographic waves were not stable for the expellation of the involved oxygen.

III. Polarographic behavior of oxidized fish oils.

In this chapter the following experiments are involved.

(1) Comparison of fresh and oxidized oils by polarograms.

(2) Increase and decrease of waves reduced in −0.4V, −1.2V and −1.9V vs S.C.E. during the oxidation.

(1) Comparison of fresh and oxidized oils by polarograms.

Fresh samples were used the same as in the former chapter II; i.e., sardine oil, tunny fish oil, whale oil, lard, beef tallow and soya bean oil.

Oxidized materials were obtained from the above-mentioned fresh fats and

Table 1. Characteristics of initial and oxidized facts and oils.
methylen blue, reduction of methylen blue[2]; Issoglio, Issoglio's test[3]; and Shiff, Shiff's test.

Sample \ Method of test	Acid value	Iodine value	Peroxide value	Aldehyde	Kries test	Methylen-blue	Issoglio	Shiff
Sardine oil	5.36	180.5	0.12	0	−	−	−	−
oxidized	15.42	156.5	115.2	72.5	+	+	+	+
Tunny fish oil	9.40	198.7	0.65	0	−	−	−	−
oxidized	17.25	153.8	90.15	45.0	+	+	+	+
Calamary oil	12.70	219.9	3.89	0	−	−	−	−
oxidized	28.72	163.4	129.69	57.5	+	+	+	+
Whale oil	1.93	141.0	0.12	0	−	−	−	−
oxidized	8.25	89.5	51.5	64.0	+	+	+	+
Lard	0.55	99.7	0.10	0	−	−	−	−
oxidized	7.24	80.5	92.10	42.0	+	+	+	+
Beef tallow	3.54	55.8	0.05	0	−	−	−	−
oxidized	11.44	48.7	72.80	39.0	+	+	+	+
Soya bean oil	0.10	137.0	0.05	0	−	−	−	−
oxidized	7.65	102.5	92.50	54.0	+	+	+	+

ils, by the two oxidized methods as follows;

(i) Stored in an open bottle for one year.—lard and beef tallow were stored

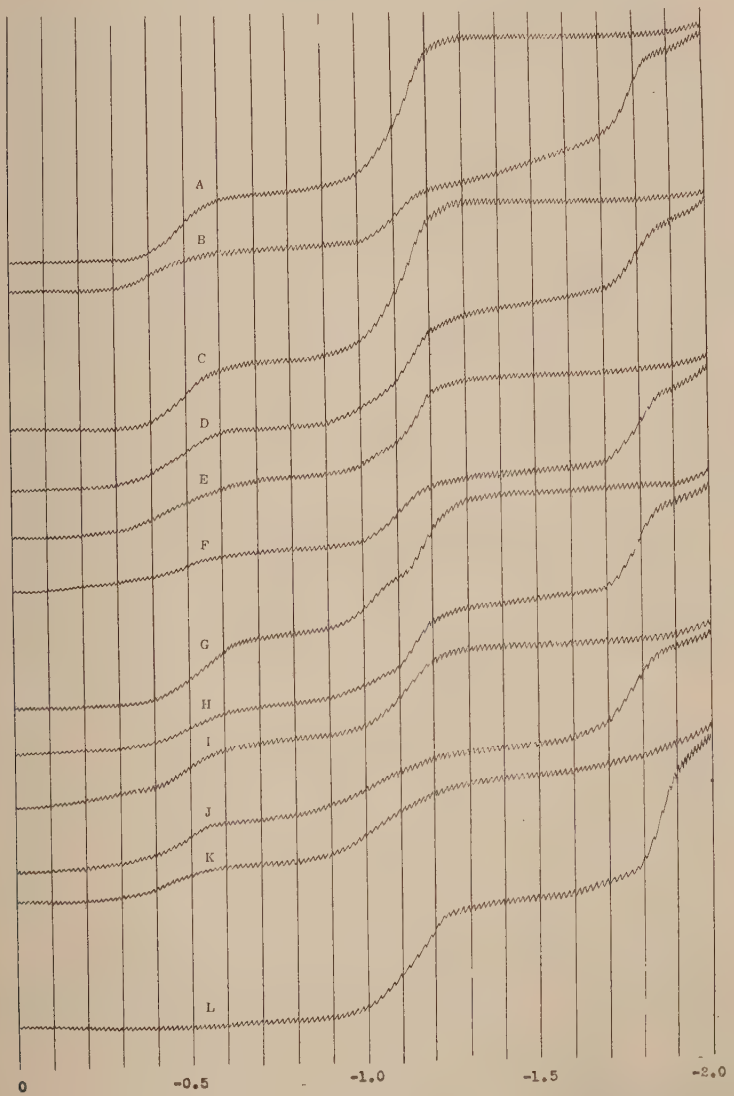

Fig. 4. Comparison of polarograms in fresh and oxidized fats and oils in 0.1N LiOH. A and B, sardine oil; C and D,tunny fish oil; E and F, calamary oil; G and H, whale oil; I and J, lard; and K and L, beef tallow. A, C, E, G, I and K were fresh fats and oils and B, D, F, H, J and L were oxidized fats and oils.

in an open bottole for one year at the room temperature, because the deteriolation of them could not be obtained in the short storage.

(ii) Oxidized by stretching on filter paper.—To sardine oil, tunny fish oil, calamary oil, whale oil and soya bean oil this method was applied.

The characteristics of initial and oxidized fats and oils were shown in Table 1.

The oxidized fats and oils showed polarographic waves, and the reduction potentials of them were −0.4V, −1.2V and −1.9V vs S.C.E.. In all oxidized samples, the same results were obtained, while in the fresh oils two waves, reduced in −0.4V and −1.2V vs. S.C.E., were observed as in the case of the oxidized oil, but the third polarographic waves having a half-wave potential of −1.9V vs S.C.E. was not observed. The comparison of polarograms in fresh and oxidized oils was shown in Fig. 4.

(2) Increase and decrease of waves reduced in −0.4V, −1.2V and −1.9V vs S.C.E. during the oxidation.

In this section, the increase and decrease of reduction waves reduced in nearly −0.4V, −1.2V and −1.9V were studied for whale oil during the oxidation.

Kries test, peroxide value, aldehyde contents, iodine number, acid value, and odor were also examined as the comparative chemical test. Whale oil was maintained at 50°C in a washing bottle and the air was bubbled through it for 9 days. Samples were taken from the bottle at intervals for polarographic method and other chemical tests. These results were shown in Table 2 and Fig. 5.

The reduction waves having the half-wave potentials of nearly −0.4V and −1.2V vs S.C.E. seemed to decreased their wave heights with increased the oil oxidation, but the detail observation could not be obtained because these waves were not so stable as the authors described in the former chapter.

Table 2. Increase and decrease of polarographic wave during the oxidation (whale oil).

Aeration, Days		0	1	2	3	4	5	7	9
Acid value		1.93	1.98	1.94	2.90	3.18	3.75	5.35	6.35
Peroxide value		0.13	0.76	3.54	27.3	34.5	52.5	71.1	44.6
Iodine value		141	141	141	133	120	120	105	91.7
Aldehyde contents		0	0.57	9.5	22	22	36	54	65
Kreis test	R	0.6	1.3	3.3	3.0	1.3	2.0	2.3	1.6
	Y	0	0	1.0	6.0	3.0	1.8	4.0	2.0
	B	0	0	0	0	0	0	1.0	0.1
Wave height (mm)		0	±	9.0	23.0	31.0	39.0	42.0	51.0
Ordor		—	—	—	—	—	±	+	++

The reduction wave at nearly −1.9V vs S.C.E. in oxidized oil, was increased the wave height with increasing oxidation.

Therefore the reduction wave seemed to be caused by an oxidation product of whale oil. Similar phenomena were also observed in the oxidation of other fats and oils. As a reason for the increase and decrease of their polarographic waves, the authors surmise that the compounds reduced at −0.4V and −1.2V

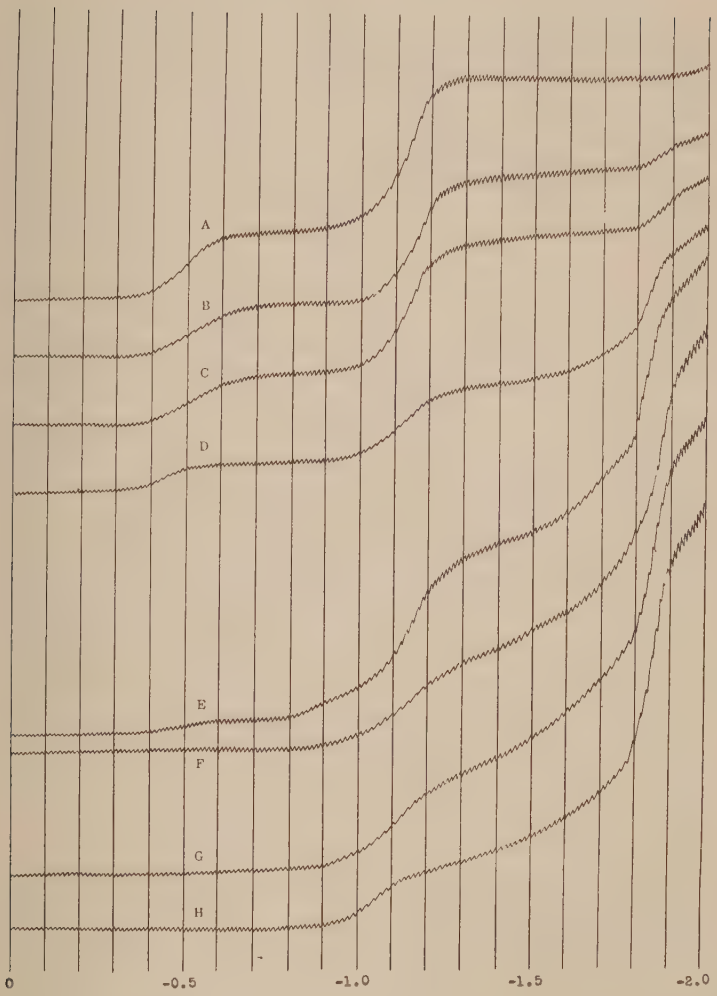

Fig. 5. Increase and decrease of the polarographic waves during the oxidation of whale oil. A, fresh whale oil; B, C, D, E, F, G and H, oxidized whale oil by aeration at 50°C for 1, 2, 3, 4, 5, 7, 9 days, respectively.

s S.C.E. are unstable intermediate compounds and then they are changed to table final compounds as showed a reduction wave at −1.9V in oxidized oils.

The animal fats differ in composition from the vegetable fats containing a reat variety of fatty acids. Both saturated and unsaturated fatty acids of the $_{20}$, C_{22} and even the C_{24} series are found in many animal fats, in addition to ne usual compounds of all fats, namely the fatty acid of the C_{16}, C_{18} series, almitic stearic and oleic acid. In the case of unsaturated acids, both the marine

and terrestrial animals have many representatives between C_{16} and C_{24}.

In the study of oxidation of fats and oils, it is necessary to investigate the deterioration of fatty acids composed these fats and oils, because the oxidative rancidity largely depends upon the presence of unsaturated fatty acids. Therefore the authors separated mixed fatty acids from fish oil and observed the polarographic characteristic of the initial and oxidized mixed fatty acids in 0.1N LiOH solution, expecting to obtaine the different polarographic waves in the initial and oxidized mixed fatty acid.

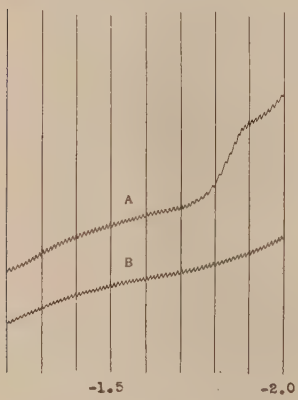

Fig. 6. Polarogram of mixed fatty acids from fish oil in 0.1N LiOH. A, oxidized mixed fatty acids; B, freshly prepared mixed fatty acid.

The mixed fatty acids was separated by a general means.

The oxidized mixed fatty acids, stretched on filter paper under the sunbeams for one month, showed a polarographic wave in 0.1N LiOH solution having a half-wave potential of −1.9V vs S.C.E..

The reduction had a resemblance to the one that the authors obtained in oxidized oil. The polarograms of freshly prepared mixed fatty acids and of the oxidized one were represented in Fig. 6. The observation of the first and the second waves, reduced at nearly −0.4V and −1.2V vs S.C.E. was abridged in this experiment, because the increase and decrease of them were not so distinct during the oxidation.

From this experiment, the polarographic waves having a half-wave potential of −1.9V, observed in oxidized oil, seemed to be responsible for the oxidation product of the component fatty acid.

IV. Polarographic observation of oxidized unsaturated fatty acids.

It is probable that the polarographic wave reduced at −1.9V vs S.C.E. in 0.1N LiOH depends upon the oxidized product of fatty acids, though several other oxidation products may be related to it. Oleic, linoleic and linolenic acids are the readily oxidizable unsaturated fatty acids and they are usually found in various fats and oils.

It was difficult to obtain linoleic acid and linolenic acid in high purity, because they were readily oxidized even at room temperature. Therefore in this experiment, oleic acid which was comparatively easy to obtain in pure state was used. Linoleic acid was also used in this experiment, but the purity was not so good as 90% of linolenic acid and 10% of ther fatty acids, therefore an satisfactory results could not be expected in the case of linoleic acid. Oleic acid was oxidized in three ways (i) aerated at 50° for two weeks, (ii) stretched on filter paper for one month (iii) elapsed in petri dish under the sunbeams for two months.

Linoleic acid was oxidized by stretching on filter paper for one month.

The oxidized oleic acid and linoleic acid showed polarographic waves as it was obversed in oxidized oils. The half-wave potential of the wave was similarly −1.9V vs S.C.E. in each case. The same observations were obtained by two oxidized methods, that is, oxidation on filter paper and aeration at 50°C. for two weeks, but in the case of the oxidation after two months in petri dish under the sunbeams, a peculiar polarographic wave was observed (Fig. 7, C). It could not be decided why the peculiar polarographic wave was appeared in such oxidized oleic acid as oxidized under the sunbeams. These polarograms were shown

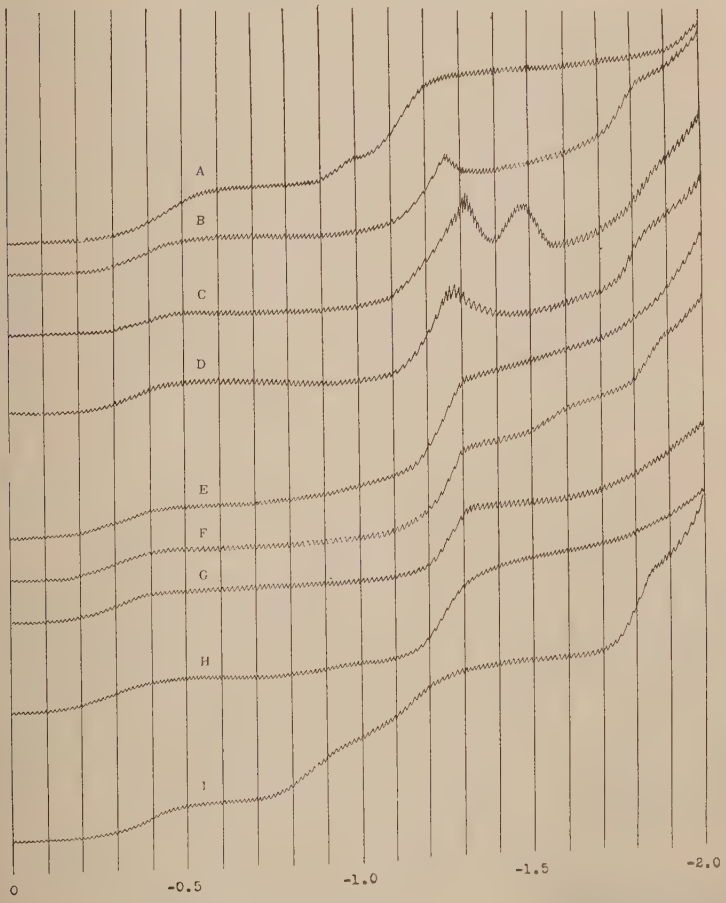

Fig. 7. Polarograms of oleic acid and related compounds in 0.1N LiOH. A, oleic acid; B, C and D, oxidized oleic acid; E, acidic carbonyl compounds separated from oxidized oleic acid; F, neutral carbonyl compounds; G, non carbonyl compounds; H, hydroxy keto stearic acid; and I, azelaic half aldeyhde.

in Figs. 7 and 8. Then the oxidized oleic acid was separated in acidic carbonyl, neutral carbonyl and noncarbonyl group and the polarographic characteristics of them were studied. The separation method was modified to the Gilard P. reagent. The polarograms of acidic carbonyl, neutral carbonyl and noncarbonyl group in 0.1N LiOH solution were represented in Fig. 7. The similar reduction waves observed in the oxidized fat and unsaturated fatty, having a half-wave potential of −1.9V vs S.C.E. were observed in the neutral carbonyl compound separated from oxidized oleic acid.

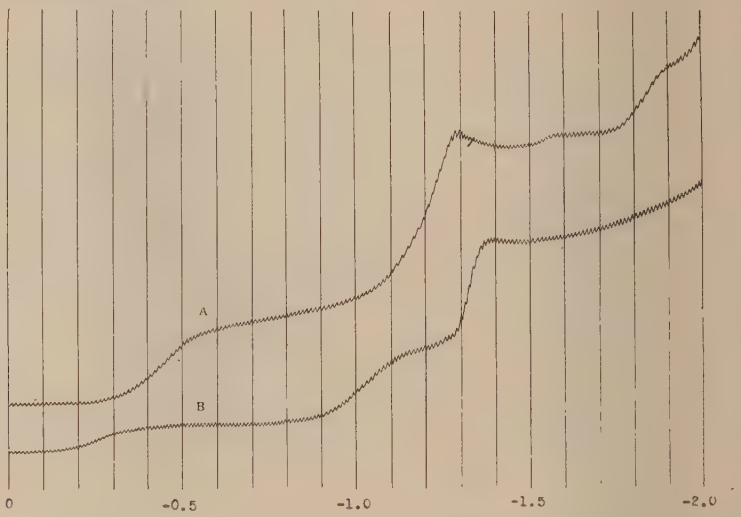

Fig. 8. Polarogram of linoleic acid in 0.1N LiOH. A, oxidized linoleic acid; and B, fresh linoleic acid.

Therefore it was considered that the polarographic wave which was reduced at the potential of −1.9V vs S.C.E. in oxidized oleic acid was caused by the neutral carbonyl compound produced during the oxidation. On the other hand, considerable compounds reduced in the oxidation of oleic acid, pure azelaic half aldehyde, nonyl aldehyde, hydroxyketostearic acid and others were studied in 0.1N LiOH solution, and the same wave was obtained in azelaic half aldehyde and nonylaldehyde as observed in the oxidized oil. The detail data will report in later paper.

V. Separation of water soluble compounds in oxidized fats and oils.

In the former chapter the authors obtained a well defined polarographic wave which was caused by the oxidation products of fats and oils.

It seems that the compound to produce the polarographic wave in oxidized fats and oils is not caused by a single substance, but several substances are related to it. Therefore in this chapter the authors describe a trial to separation of the water-soluble compounds produced during the oxidation of fats and

oils by the systematic steam distillation.

The polarograms were obtained on each distillate and residue with several electrolytic solutions and the other chemical tests were also made.

Material and method

1) Sample. The fats and oils used were oxidized sardine oil, tunny fish oil, calamary oil, whale oil, lard, beef tallow and soya bean oil.

2) Steam distillation. The schematic diagram, Fig. 9, represented the method for the separation of water soluble compounds from oxidized fats and oils.

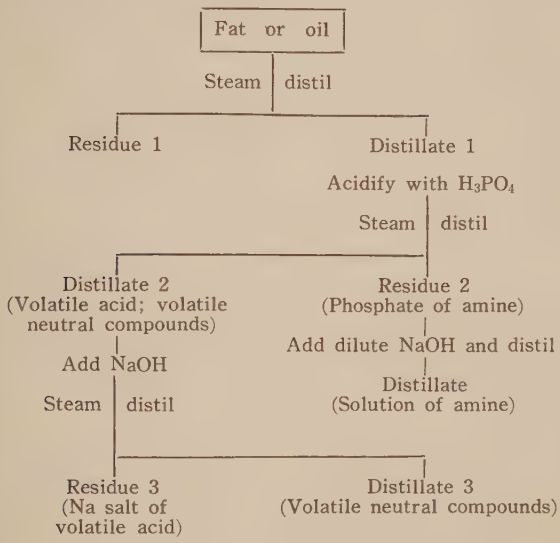

Fig. 9. Schematic diagram of separation of water soluble compounds.

a) About 50 ml. of oxidized oil was placed in a 500 ml round-bottle flask arranged for steam distillation. By means of steam distillation from 50 to 60 ml. of distillate 1 was collected.

b) Distillate 1 was acidified with phosphoric acid and steam-distilled to yield 40 to 50 ml. of distillate 2. Residue 2 consisting of the phosphate of the amine; was made alkaline and the amine was distillated.

c) Distillate 2 was made just slightly alkaline with dilute sodium hydroxyde solution and was steam-distilled again, and 30 to 40 ml. of distillate 3 was collected. In general, residue 3 is composed of the sodium salt of the acidic compound. And distillate 3 contains volatile neutral compound, alcohols aldehydes and ketones.

3) Supporting electrolyte solution.

The supporting electrohyde solution used in this experiment was mainly 0.1N LiOH solution, and others were McIlvaine's buffer solution of pH 7.0, 6.0 and 5.0, 0.1N NH_4Cl solution, and 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide solution.

(4) Chemical test

Other chemical tests through the polarographic investigation were shiff's test, and Brady's reaction.

(i) The polarograms of distillates and residues in alkaline (0.1N LiOH) medium.

Table 3. Characteristics of distillates and residues of fresh and oxidized oils. Methylen blue, reduction of methylen blue[2]; Issoglio, Issoglio's test[3]; Shiff, Shiff's reaction; Brady, Brady's reaction; 2,4, D.N.P.H., 2.4, dinitrophenyl hydrazone; A, reduction wave in 0.1N LiOH; B, C and D, McIlvaine's buffer solution of 5.0, 6.0 and 7.0; E, 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide; D, disillate and R., residue.

Method of test / Sample	Methylen-blue	Isso-glio	Shiff	Brady	λ_{max}, 2,4, D.N.P.H.	Polarographic reduction wave A	B	C	D	E
oxidized sardine oil										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356mμ	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)
oxidized tunny fish oil										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)
oxidized calamary oil										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)
oxidized whale oil										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)

Table 4. Characteristics of distillates and residues of fresh and oxidized fats and oil. Methylen blue, reduction of methylen blue[2]); Issoglio, Issoglio's test[3]); Shiff, Shiff's reaction; Brady, Brady's reaction; 2,4, D.N.P.H., 2, 4 dinitrophenyl hydrazone; A, Reduction wave in 0.1N LiOH; B, C and D, McIlvaine's buffer solution of 5.0, 6.0 and 7.0; E; 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide; D., distillate and R., residue.

Sample \ Method of test	Methylen-blue	Isso-glio	Shiff	Brady	λ_{max}, 2, 4, D.N.P.H.	Polarographic reduction wave A	B	C	D	E
oxidized lard										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)
oxidized beef tallow										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)
oxidized soya bean oil										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)

Table 5. Characteristic of distillates and residues of fresh and oxidized fatty acids. Methylen blue, reduction of methylen blue[2]); Issoglio, Issoglio's test[3]); Shiff, Shiff's reaction; Brady, Brady's reaction; 2, 4, D.N.P.H., 2, 4 dinitrophenyl hydrazone; A, Reduction wave in 0.1N LiOH; B, C and D, McIlvaine's buffer solution of 5.0, 6.0 and 7.0; E, 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide; D., distillate and R., residue.

Sample \ Method of test	Methylen-blue	Isso-glio	Shiff	Brady	λ_{max}, 2, 4, D.N.P.H.	Polarographic reduction wave A	B	C	D	E
oxidized oleic acid										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	356	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	− (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)

Table 5. (Continued)

Sample \ Method of rest	Methylen-blue	Isso-glio	Shiff	Brady	λ_{max}, 2, 4, D.N.P.H.	Polarographic reduction wave A	B	C	D	E
oxidized linoleic acid										
D. 1 (fresh)	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 2 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
D. 3 〃	+ (−)	+ (−)	+ (−)	+ (−)	357	+ (−)	− (−)	− (−)	− (−)	+ (−)
R. 1 〃	+ (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 2 〃	+ (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	− (−)
R. 3 〃	+ (−)	− (−)	− (−)	− (−)	—	− (−)	− (−)	− (−)	− (−)	+ (−)

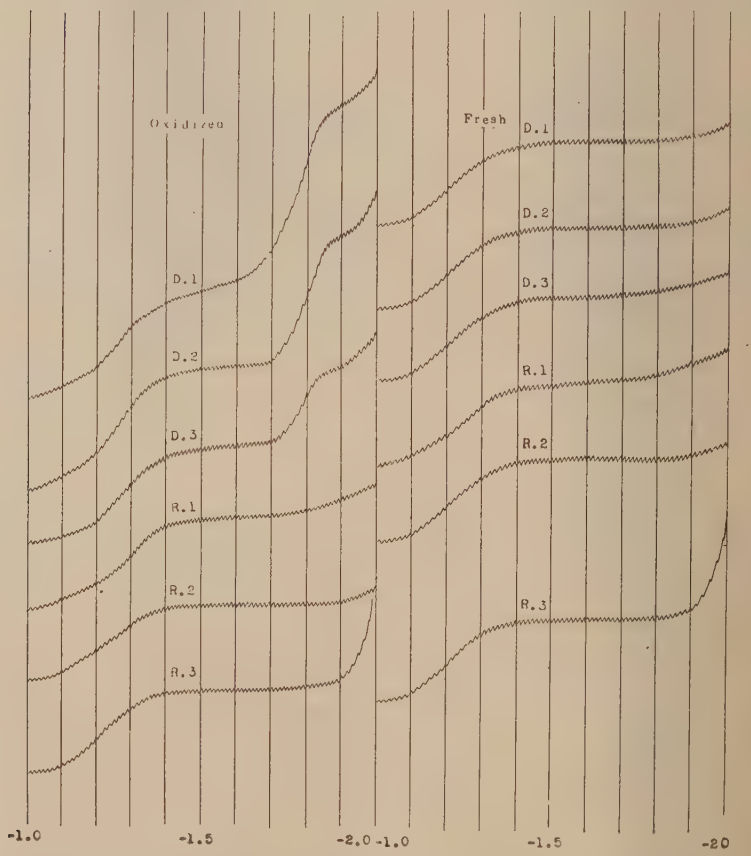

Fig. 10. Polarograms of distillates and residues in 0.1N LiOH.—Sardine oil. D., distillate; and R., residue.

A division of the distillate 1, 2 and 3 and residue 1, 2 and 3 was used to the polarographic method, with the supporting electrolytic solution of 0.1N LiOH. These solutions were adjusted to pH 12.5.

The other divisions of the distillates and residures were used for Shiff's test and Brady's reaction. The polarograms of distillates and residues were separated from oxidized sardine oil, tunny fish oil, calamary oil, whale oil, lard, beef tallow, soya bean oil, oleic acid and linoleic acid, and the results of chemical tests were represented in Tables 3, 4 and 5 and Figures of 10~16.

The polarographic wave having a half-wave potential of −1.9V vs S.C.E. was observed in distillate 1, 2 and 3, and not in residue 1, 2 and 3. In this experiment the same observations were obtained as in all oxidized fatty materials.

The polarograms of distillate 2 and 3, and residue 1, 2 and 3 separated from

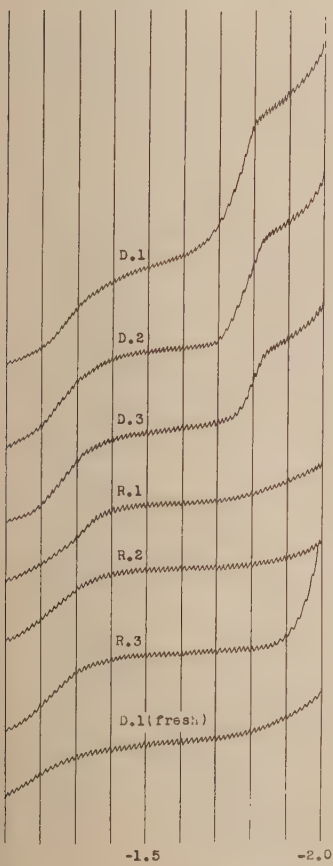

Fig. 11. Polarograms of distillates and residues in 0.1N LiOH.—Tuna fish oil. D., distillate; and R., residue.

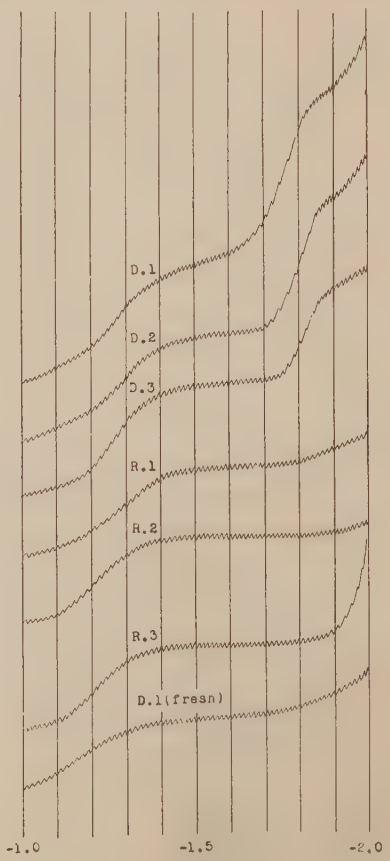

Fig. 12. Polarograms of distillates and residues in 0.1N LiOH—Calamary oil. D., distillate; and R., residue.

fresh fatty materials were not described in Figures. In these observations the polarographic wave reduced at −1.9V vs S.C.E. seemed to be responsible for the volatile substance produced during the oxidation of fats and oils and the compounds seemed to belong to aldehyde or ketone group. The comparative investigation between the characteristics of the wave and the further studied characteristics of pure compounds, aldehyde, ketone and others, are described in "discussion".

(ii) The polarograms of distillates and residues in neutral and acidic media.

The polarographic characteristics are always different in each supporting electrolyte, therefore this section describes the polarographic behavior of the distillates and residues in neutral and acidic media.

McIlavine's buffer solution of pH 7.0, 6.0 and 5.0, 0.1N NH_4Cl, and 0.1N

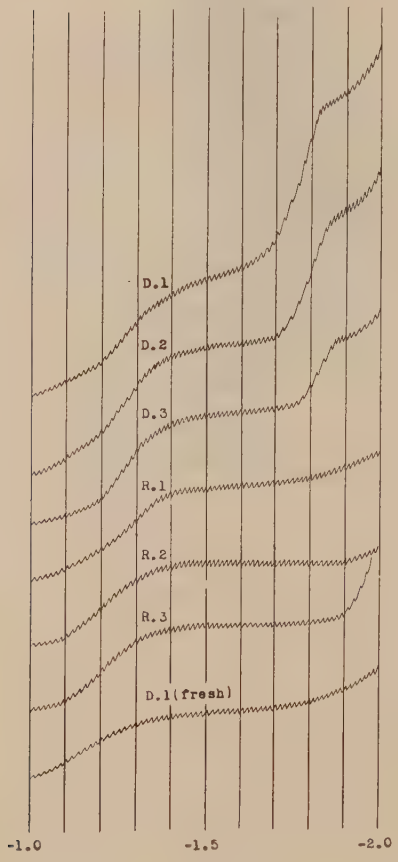

Fig. 13. Polarograms of distillates and residues in 0.1N LiOH—Whale oil. D., distillate; and R., residue.

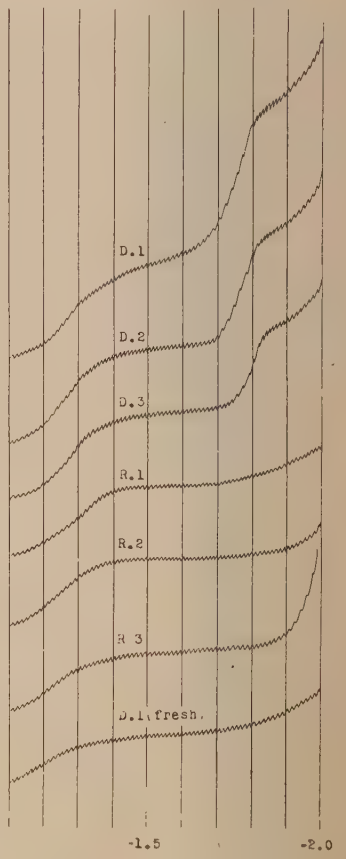

Fig. 14. Polarograms of distillates and residues in 0.1N LiOH—Lard. D., distillate; and R., residue.

LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide solution were added to distillate 1, 2 and 3 and residue 1, 2 and 3, and the polarograms were studied. These solution were carefully adjusted to pH value of 7.0, 6.0 and 5.0 in the case of buffersolution.

These polarograms were represented in Fig. 17.

Distillate 1, 2 and 3 and residue 1, 2 and 3 did not show polarographic waves in McIlvaine's buffer solution of pH 7.0, 6.0 and 5.0.

Distillate 1, 2 and 3, and residue 3 showed a polarographic wave at −1.8 V vs. S.C.E. in 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetra methyl ammonium iodide solution, and the residue 1 and 2 did not show the polarographic wave. It seemed that the wave was responsible for the aliphatic acid reduced during the oxidation.

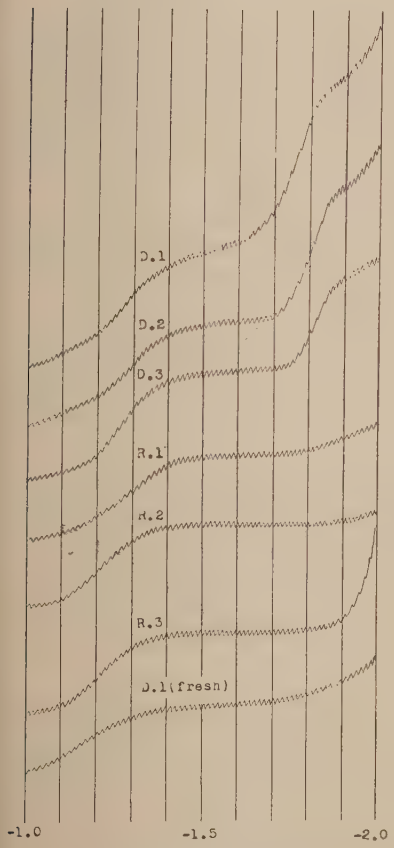

Fig. 15. Polarograms of distillates and residues in 0.1N LiOH—Soya bean oil. D., distillate; R., residue.

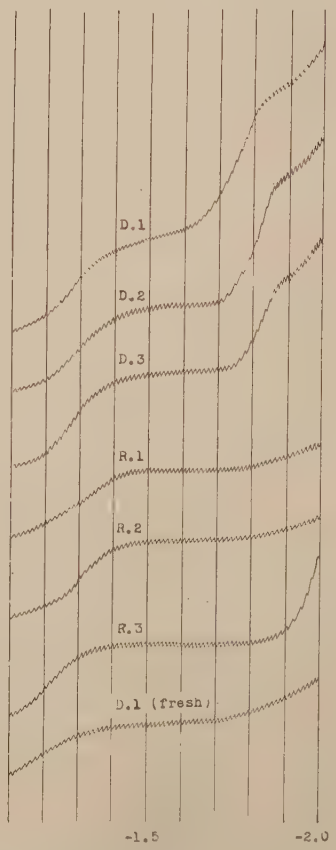

Fig. 16. Polarograms of distillates and residues in 0.1N LiOH—Linoleic acid. D., distillate; R., residue.

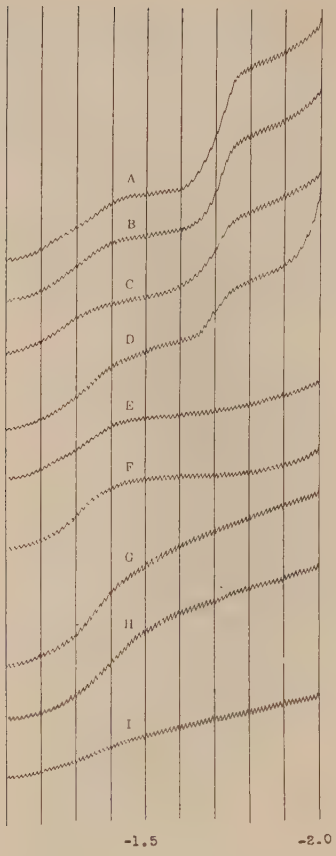

Fig. 17. Polarograms of distillates and residues in acidic and neutral medium. Oxidized sardine oil. A, distillate 1; B, distillate 2; C, distillate 3; D, residue 3; E, residue 2; F, residue 1, in 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide. G, distillate 1 in McIlvaine's buffer solution of pH 5.0; H, distillate 1 in McIlvaine's buffer solution of pH 6.0; and I, distillate 1, in McIlvaine's buffer solution of pH 7.0.

Discussion

The decomposition products of fats and oils can be devided into polarographically reducible and non reducible categories. And by many investigators the polarographic studies on the pure compounds concerning oxidation products of fats and oils, such as peroxides, aldehydes, ketones, fatty acids etc. have been reported.

Here, the polarographic studies of them are quoted and the authors try to compare the polarographic characteristics of them with the waves which the authors observed in oxidized oils. In this chapter, the discussion on the polarographic waves which were observed in fresh and oxidized oils, having the half-wave potentials of nearly −0.4V and −1.2V vs. S.C.E. was abridged because these waves were not regular or well defined enough to assign half-wave potentials.

(1) Aliphatic acid.

Simple aliphatic acids give a wave at the dropping mercury electrode that is due to the discharge of the hydrogen ions[18]. No wave is obtained with the esters (ethyl acetate)[19].

The half-wave potential becomes more negative when the concentration of the acid increases. Values obtained for number of acids in 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide solutions in concentrations of 1–10 millimoles per liter are given in Table 4[18].

The reduction wave that the authors obtained in the oxidized fats and oils and distillate separated from them was more negative than the pure aliphatic acids.

By the authors experiment, simple aliphatic acids were reduced at the same potential of 1.75∼1.85V vs S.C.E. in 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide solution as presented in Table 4. But simple aliphatic acid did not reduce polarographically at about −1.9V in 0.1N LiOH

solution. Therefore the polarographic wave showing the half-wave potential of −1.9V vs S.C.E. seemed not to be caused by the simple aliphatic acid.

Table 6. Polarographic behavior of simple aliphatic acid.

Acid	Conc., millimoles per liter	−E1/2 (S.C.E.)
Formic	1–10	1.74–1.85
Acetic	2–15	1.76–1.86
Isobutyric	3–25	1.81–1.87
Isovaleric	4–27	1.75–1.82

(2) Unsaturated aldehydes.

Unsaturated aldehydes in which the aldehyde group is conjugated with one or more double bonds will give two reduction waves. Acrolein and crotonaldehyde are the most probable unsaturated aldehydes to be found in the oxidized fats and oils.

(a) Acrolein has been studied in various buffers and it has been found to give two waves[20]. The following half-wave potentials are reported for the first wave.

pH	4.8	5.8	7.0–11.0
E1/2 Volt	−0.83	−0.98	−1.04

A value of −1.44V is obtained for the second wave in buffers with pH 8.7–11.0. In more acidic buffers the second wave is obscured by the residual current.

The diffusion current of the first wave decreases with the increase in pH whereas that of the second wave increases with the increase in pH.

Gerber et al[21]. suggest that acrolein may be determined in acid (pH 3–4) or weakly alkaline solution (pH 8). In the presence of saturated aldehydes, acid solutions are recommended, for the saturated aldehydes are not reduced in this range. If peroxide are present the determination is best carried out at pH 5–6 to avoid interference with peroxide value.

In the authors experiments, distillate 1, 2 and 3 separated from oxidized fish oil did not show the polarographic wave in acidic buffer solution (pH 5.0, 6.0, 7.0). Therefore the observed reduction wave having a half wave potential of −1.9V vs. S.C.E. in 0.1N LiOH, was not caused by acrolein.

(b) Crotonaldehyde[22]. Crotonaldehyde behave the same as acrolein and gives two waves[23] with half-wave potentials of −1.37V (1st wave) and −1.80V (2nd wave) in 0.2N tetramethyl ammonium hydroxide, and 1.25 (1st wave) and −1.55V (2nd wave) in 0.1N ammonium chloride solution (the last two potential have been referred by them to the S.C.E.). The reduction potential of crotonaldehyde was similar to the potential that the authors obtained in oxidized oil. The polarographic investigation of pure crotonaldehyde was not yet examined in several supporting electrolyte, therefore satisfactory identification could not be obtained in the present study.

(3) Aldehydes and ketones.

(a) Aldehyde. Half-wave potentials (vs S.C.E.) are reported by Elving and Rutner for acetaldehyde of −1.89V in lithium acetate-acetic acid buffer of pH 6.8, −2.04V in unbuffered lithium acetate solutions (pH 8.8), and −1.89V in a 0.06M LiOH −0.05M LiCl solution (pH 12.7)[24].

Values of several other aliphatic aldehydes are listed in Table 7.

Table 7. Polarographic reduction potential of aliphatic aldehydes.

Name	Half-wave potential[a]	Reduction potential (N.C.E.)
Formaldehyde	−1.65[e]	−1.38[b]
Acetaldehyde	−1.68[e]	−1.60[c]
Propion aldehyde	−1.92	−1.60[c]
n-Butyraldehyde	−1.90	——
iso-Butyraldehyde	−1.91	——
n-Valeraldehyde	——	−1.36 (molar)[d]
n-Capronaldehyde	−1.98	——
n-Heptaldehyde	−1.90	——

a) H. Adkins and F.W. Cox, J. Am. Chem. Soc., 60, 1151 (1938). They used 0.001M solutions of aldehyde in 0,1N tetramethylammonium hydraxide; these authors did not measure the anode potential, but it was probably close to −0.2V (S.C.E.).

b) A. Winkel and C. Proske, Ber., 69, 693 (1936). They used 0.1M formaldehyde (partly polymerized) in 0.1M ammonium chloride.

c) Same as b), except with 0.001M aldehyde solution.

d) M. Shikata and I. Tachi, Mem. Coll. Agr. Kyoto Imp. Univ., 4, 9 (1927); 0.0187 M aldehyde solution in 0.1N ammonium chloride. In 0.1N hydrochloric acid they found −1.20V., in 0.1N potassium chloride −1.35V., in 0.1N sodium hydroxide −1.82V.

e) R. Bieber and G. Trumpler, Helv. Chem. Acta, 30, 2000 (1947); 31, 5 (1948). The supporting electrolyte was 0.1M LiOH, and the half-wave potentials were represented vs N.H.E.

Half-wave potential of these aliphatic aldehyde that the authors studied in 0.1N LiOH solution was nearly −1.9V vs S.C.E. as descrided above.

It has been also known by further investigators the aliphatic aldehyde did not reduce in the acidic medium. In the former chapter of the authors experiment, distillate 1, 2 and 3 distilled from oxidized oils did not show the polarographic wave in acidic McIlvaine's buffer solution. Therefore the polarographic wave of which half-wave potential is −1.9V vs S.C.E. in oxidized fat and oil seemed to be responsible for aldehyde group.

(b) Aliphatic ketones. Most of the aliphatic ketones cannot be determined directly by the polarographic method. Negative results[25] of many aliphatic aldehydes are reported on solutions containing hydrochloric acid, lithium chloride, or sodium hydroxide as the inert electrolyte.

Therefore the discussion of them was abridged.

(c) Others.—Biacetyl. Biacetyl gives two wave of about equal height in 0.1N NH_4Cl at −0.84 and −1.59V vs S.C.E.. In a 0.1N KCl solution the half-wave potential for the first wave is −0.83V vs S.C.E.[26]. In 0.1N LiCl solution

reduction potentials of −0.81 and −1.93V. were obtained which in neutral and alkaline media shifted to less negative potential in the presence of divalent cations, such as calcium and barium[27].

The effects of the concentration of biacetyl upon reduction potentials and the heights of the waves have been studied systematically in various solution[28].

The reduction wave that the authors obtained in oxidized oil, is similar to biacetyl, but the satisfactory identification could not be described by the polarographic method only.

From these comparative discussion, the polarographic waves reduced in oxidized oil, having a half-wave potential of −1.9V vs S.C.E. seemed to be responsible for aliphatic aldehyde.

The authors studies on the 2, 4-dinitrophenylhydrazone obtained from distillation of oxidized oil by spectrophotometry, paperchromatography and on the infra-red spectrometry of oxidized oils. These results have not been reported, but several aldehydes and aldehyde like compounds were obtained in the distillate of oxidized oil. Therefore the polarographic wave, described in this report, may be responsible for aldehydes and aldehyde like compounds reduced during the oxidation of fats and oils.

Summary

The polarographic method was applied to the studies on the oxidation of fish oil, and the following investigations were carried out.

(a) Polarographic investigations of fresh fish oils.

For samples; sardine oil, tunny fish oil, calamary oil, whale oil, lard, beef tallow, and soya bean oil were used. These oils and fats reduced two polarographic waves, and the half-wave potential was −0.4V and −1.2V vs S.C.E. but these were not so defined waves that the wave heights were not stable to the bubbling N_2, H_2 or CO_2 gas to expel of involved oxygen.

(b) Polarographic investigation of oxidized fish oils.

The different polarographic wave reduced in −1.9V vs. S.C.E. was found in oxidized fish oils and was not observed in fresh oils. The height of this wave increased as the oxidation of oil increased. The investigation was carried out by the polarographic method as compared with other chemical tests.

(c) Polarographic investigation of oxidized unsaturated fatty acid.

Oxidized oleic acid showed polarographic waves, one of them, having a half-wave potential of −1.9V vs S.C.E., was similar to the one observed in oxidized fish oils. On the other hand, many compounds which will be produced in the oxidation of oleic acid, i.e azelaic halfaldehyde, nonyl aldehyde and other, were studied, and the same waves were obtained in azelaic halfaldehyde and nonyl aldehyde as obtained in the oxidized fish oils. Oxidized oleic acid stretched on filter paper for two months under the sunbeams showed a peculiar polarographic waves, the detail observation was not carried out in this experiment. In the case of linoleic acid the −1.9V wave was the same as the case of oleic acid, but the linoleic acid used had not so high purity and so the results was doubtful.

(d) Polarographic studies on the distillate of oxidized fish oil.

The polarograms were studied on the distillate of oxidized fish oils, by systematic steam distillation, in several electrolytic solution 0.1N LiOH; and Mac Ilvaine's buffer solution of pH 7.0, 6.0 and 5.0 and 0.1N LiCl, 0.1N Li_2SO_4 and 0.05N tetramethyl ammonium iodide.

The compound reduced in about −1.9V vs S.C.E. seemed to be caused by the aldehyde group reduced during the oxidation of fish oil.

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This study was supported in part by a Grant in Aid for Departmental Scientific Research from the Ministry of Education and Fisheries Agency to which the offers their hearty thanks.

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EXPERIMENTS ON THE FREEZING OF FISH BY THE AIR-BLAST FREEZER

Junkichi Nagaoka, Shigetake Takagi and Sachio Hotani

(Received Oct. 15, 1955)

In Japan recently some air-blast freezers and so-called semi-air-blast freezers which are just like the Heckermann freezers have been installed.

The chief object to install the air-blast freezers are rather to promote the production of frozen fish by shortening the freezing period than to improve their quality. Therefore the relation between the cold air speed and the freezing period is important for the design of such freezers.

In these experiments various kind of fish were frozen in the cabinet type air-blast freezer, especially made for these experimental purposes. From these experiments, it was found that the cold air velocity of above 5 m/s was of little use for the shortening of the freezing period of fish. But the cold air velocity of above 5 m/s sometimes changes much the skin colour of fish and rather slow air velocity such as 2 to 3 m/s is prefered for such freezers in Japan now.

The other reason to use rather low air speed is as follows. By using high air velocity, we can freeze the fish thrice a day but night work can not be avoided, which makes the labor cost high. At such low air speed as 2 to 3 m/s, we can reduce the freezing period to half of that of the sharp freezers where no air-blast is used. Thus we can freeze fish twice a day by air-blast freezers, whereas it can be done once a day by sharp freezers. This method is believed now in Japan to be the most economical one for the freezing of fish.

The air-blast freezer, we used for these experiments, is shown in Fig. 1. The freezing chamber can be separated into three parts and the air velocity of each parts can be controlled by dampers, and by change of the revolutions of the blower. Thus we can freeze the fish at three different air speed simultaneously. In the freezing chamber, some cooling pipes are arranged so as to make shelves on which the fish will be frozen, just like the ordinary sharp freezers.

The cabinet is refrigerated by a small two-stage ammonia refrigerating machine and can be cooled to about $-45°C$ when the fan is running.

Air velocities in the freezing chamber were measured by a small Robinson type annemometer with electric contact which enables us to measure the air velocity from outside the cabinet by lighting the lamp whenever the wind wheel revolves a certain number of revolutions.

The temperature of the cold air and the fish were measured by copper-constantan thermo-couples (B.S. No. 31 wire was used) and a potentiometer.

Fig. 2 is one of the freezing curves of the fish, we have got in these experiments. From these datas, we have calculated the heat transfer rate at the surface of the whole fish by Plank's equation which is somewhat modified so that it can be used for the fish whose section is rather elliptic than round.

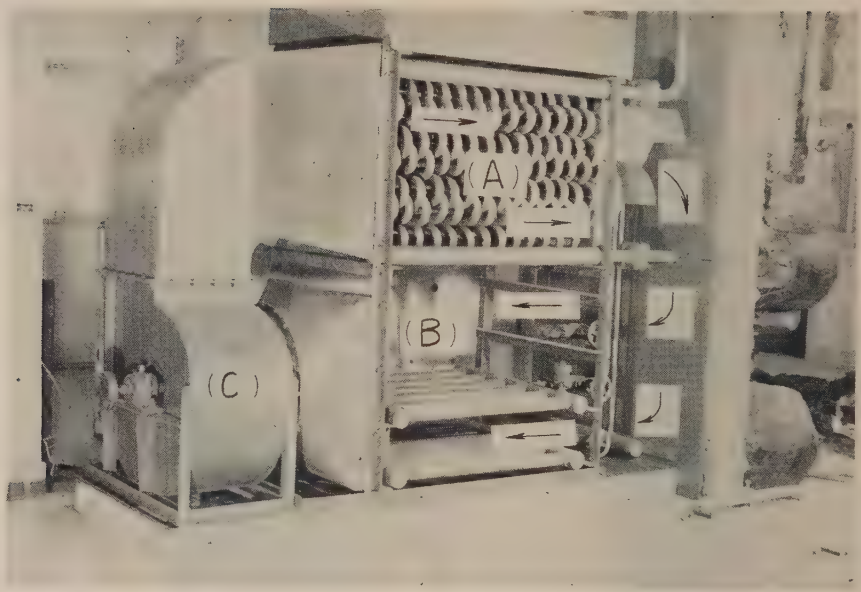


Fig. 1. Internal view of air-blast freezer. Height 1580 mm, length 1960 mm, width 720 mm. Outside wall removed. A: Cooling coil, B: Freezing chamber, C: Blower.

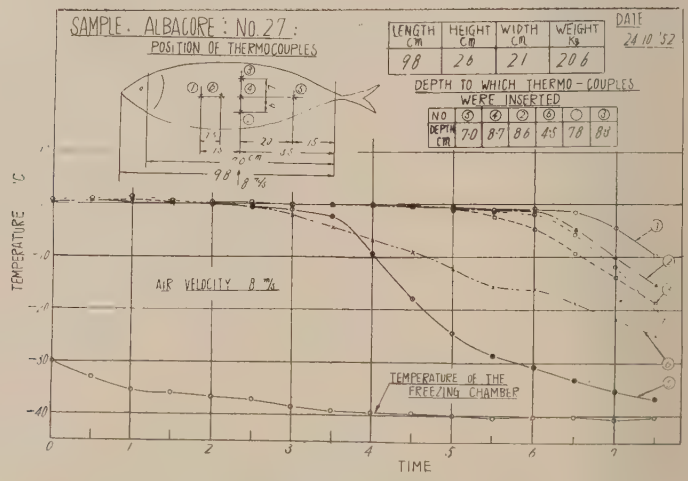

SAMPLE. ALBACORE : NO 27 :
POSITION OF THERMOCOUPLES
LENGTH CM 98
HEIGHT CM 26
WIDTH CM 21
WEIGHT Kg 2.06
DATE 24.10.'52
DEPTH TO WHICH THERMO-COUPLES WERE INSERTED
NO ⑤ ④ ② ⑥ ○ ①
DEPTH CM 7.0 8.7 8.6 4.5 7.8 8.8
AIR VELOCITY 8 M/S
TEMPERATURE OF THE FREEZING CHAMBER
TEMPERATURE °C
TIME

Fig. 2. Freezing curve of albacore.

The modified equation for the elliptic section, that is, for the whole fish can be written as follows:

$$Z=(1+0.008\theta a)\frac{\rho}{16\lambda\vartheta}De\left(De+\frac{4\lambda}{\alpha}\right)$$

where Z=freezing period. *hr*

θa=initial temperature of fish °C

ρ=latent heat of freezing, which is given by

$$[80+0.5(\theta_b-\theta_m)]\gamma\cdot\xi\cdot\delta\ .$$

in which θ_b is the mean freezing temperature °C.

θ_m the mean temperature of frozen fish °C, γ the specific weight of fish kg/m³, ξ the water content of fish and δ the freezing rate of water content, which is given by

$$\delta=1-\frac{\theta_f}{\theta_m}$$

where θ_f is the initial freezing temperature °C

λ=heat conductivity of frozen fish kcal/m.h.°C.

α=coefficient of heat transfer rate at the surface of the fish.

ϑ=temperature difference between the mean freezing temperature of fish and the temperature of cold air °C.

De=equivalent diameter of fish which is given by $2ab/(a+b)$ where a is the height of fish, and b its width m.

We first calculated the approximate value of α from the freezing period taken to freeze the fish at its centre to −5°C, taking $\theta_m=-5$°C. (in the case of cuttle fish −10°C). Using the approximate value of α, thus obtained, we calculated θ_m, δ and ρ. We repeat the calculation of α by using θ_m, and ρ obtained above. The values of α we got in these experiments are plotted against the air velocity in Fig. 3. It was found that at the same air velocity, the heat transfer rate of large fish was smaller than that of small fish.

This tendency coincides with the equation of heat transfer in the air flow at the right angles to single cylinders, which is given by $\alpha\frac{D}{\lambda}=0.24(Re)^{0.6}$ where D is the diameter of cylinder and Re, the Reynold number. We have corrected α

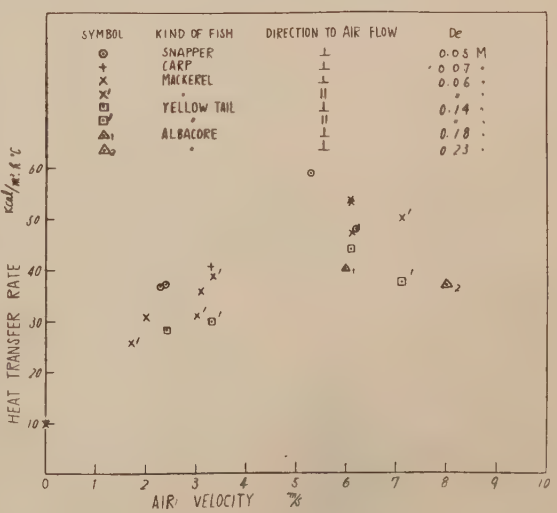


Fig. 3. Relation between heat transfer rate of whole fish and air velocity.

obtained in these experiments by the above equation to that when *De* is equal to 0.06 m, assuming the heat transfer rate by radiation equals to 3 kcal/m².h.°C. This corrected values are plotted in Fig. 4.

From Fig. 4 it was found that the heat transfer rates were nearly proportional to the 0.6th power of air velocity and inversely proportional to the 0.4th power of the equivalent diameter of fish. In Fig. 4 also is shown the theoretical heat transfer rate which is given by the calculated value from the above equation plus 3 kcal/m².h.°C., the assumed heat transfer rate by radiation.

The heat transfer rates we obtained in these experiments were about 25% greater than those calculated. This is partly due to the evaporation of water at the surface of fish. From our calculation, if the fish skin is wholly wet, the heat transfer rate will be increased by about 20% due to the evaporation of water.

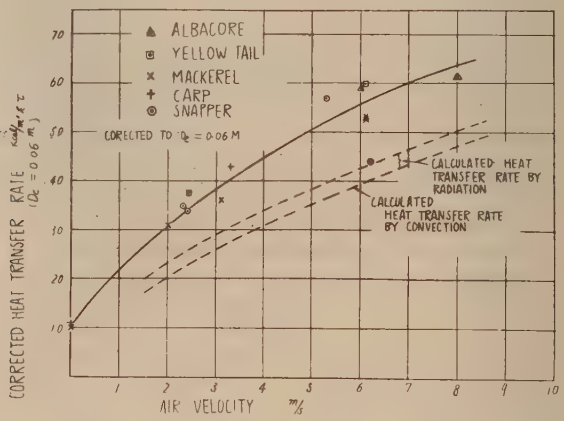


Fig. 4. Relation between corrected heat transfer rate of whole fish and air velocity.

When the fish was laid parallel to the air stream, the heat transfer rate was about 10% smaller than those when the fish were laid across the air stream.

To calculate the heat transfer rate of panned fish, we used the following equation according to Dr. Plank.

$$Z=(1+0.008\theta a)\frac{\rho'}{\vartheta}\left(P\frac{ho}{\alpha}+R\frac{ho2}{\lambda'}\right)$$

where ho = thickness of the panned fish m.

P, R = coefficient which depend on the ratio of the length to the thickness and that of the width to the thickness.

ρ' = latent heat of freezing which is given by $[80+0.5(\theta_b-\theta_m)]\gamma\cdot\xi\cdot\delta\cdot\nu$ where ν is the void factor which is given by the actual weight of panned fish divided by the weight when the whole volume of the pan is filled with fish.

λ' = heat conductivity of panned fish kcal/m.h.°C.

In the panned fish there were some voids between the fish, therefore the heat conductivity were corrected for these voids. To calculate the heat conductivity of panned fish λ', we assumed that the fish were replaced by the figure as shown in Fig. 5. And the heat was only conducted through the part of meat, that is the hatched part in Fig. 5.

The heat conductivity of panned fish thus calculated, is shown in Fig. 6.

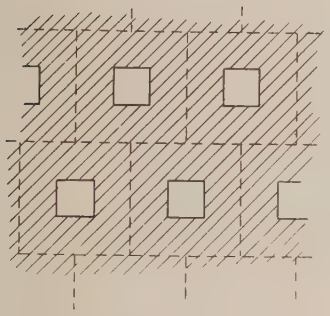


Fig. 5. Equivalent figure of panned fish.

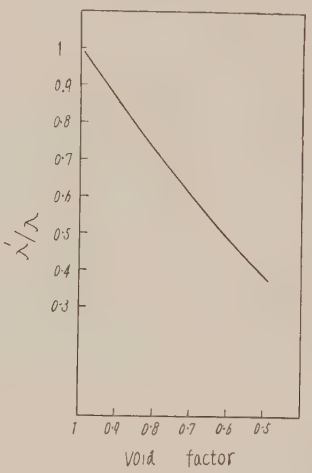


Fig. 6. Relation Between λ'/λ and the void factor. λ', heat conductivity of panned fish; λ, heat conductivity of whole fish.

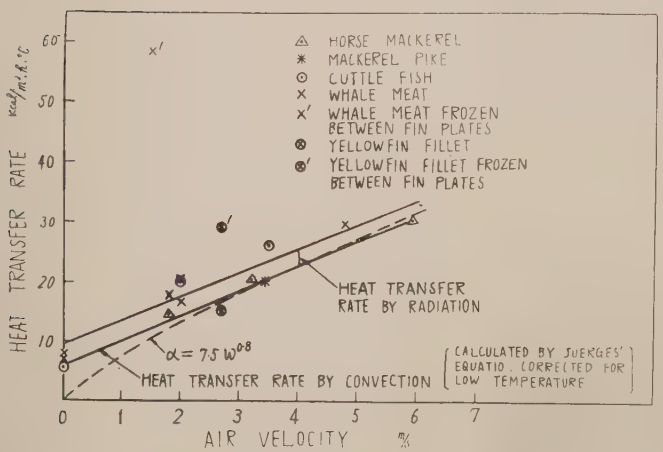


Fig. 7. Relation between heat transfer rate of panned fish and air velocity.

In Fig. 7 the heat transfer rates obtained in these experiments are plotted against the air velocity. It was found that these value approximately coincide with those obtained by Dr. Plank, that is $\alpha=7.5w^{0.8}$, where w is the air velocity in m/s. In this figure, also are shown the heat transfer rates when the fin plates as shown in Fig. 8 were attached on both sides of the fish during the freezing. It shows a remarkable increase of the heat transfer rate when the freezing fish contacts well with the plates. In calculating these heat transfer rates, we assumed the values of λ, θ_f, θ_b, ξ and γ as shown in Table 1.

Fig. 8. Fin plate. Height of fin 50 mm; pitch of fin 15 mm; thickness of fin 0.3 mm.

Table 1. The assumed values of θ_f, θ_b, ξ, γ and λ

Kind of Fish	θ_f °C	θ_b °C	ξ	γ kg/m³	λ kcal/m.h.°C
Mackerel	−1	−2	0.70	980	1.4
Yellowtail	−1.25	−2.5	0.70	980	1.4
Yellowfin	−1.25	−2.5	0.75	990	1.5
Albacore	−1.25	−2.5	0.75	990	1.5
Horse mackerel	−1	−2	0.75	990	1.5
Mackerel pike	−1.25	−2.5	0.70	980	1.4
Whale	−1	−2	0.55	1030	1.1
Snapper	−1.5	−3	0.75	990	1.5
Carp	−0.75	−1.5	0.70	980	1.4
Cuttle fish	−2.25	−4.5	0.80	980	1.6

We put the pan which was filled with water in the freezing room. The heat transmission rates at the bottom surface of the pan and the upper water surface were calculated from the thickness of ice frozen in the pan. These heat transmission rates are plotted in Fig. 9. The heat transmission rate at the bottom surface of the pan coincides well with the equation of heat transmission in the flow of air parallel to the plane, but that at the surface of water is greater due to the evaporation of water and the roughness of the surface.

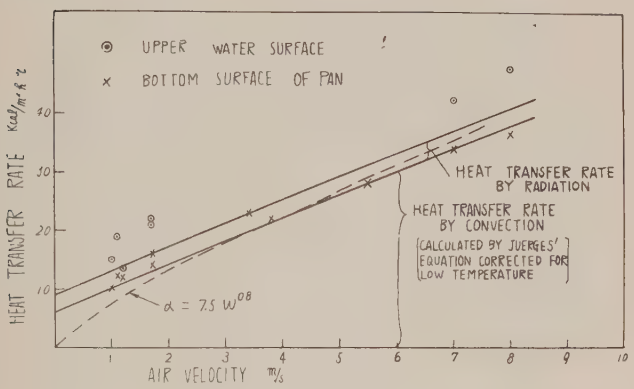

UPPER WATER SURFACE
BOTTOM SURFACE OF PAN
HEAT TRANSFER RATE BY RADIATION
HEAT TRANSFER RATE BY CONVECTION
CALCULATED BY JUERGES' EQUATION CORRECTED FOR LOW TEMPERATURE
α = 7.5 W^0.8
HEAT TRANSFER RATE Kcal/m²h°C
AIR VELOCITY m/s

Fig. 9. Water-freezing test.

In Fig. 10, is shown the difference of temperature distribution in the panned fish which was put in a pan made of the galvanized iron plate and that of the fish when put in the wooden box, made of wood plates of 12 mm in thickness. The freezing period of the panned fish put in the wooden box was about 50% longer than that of the panned fish put in an ordinary pan.

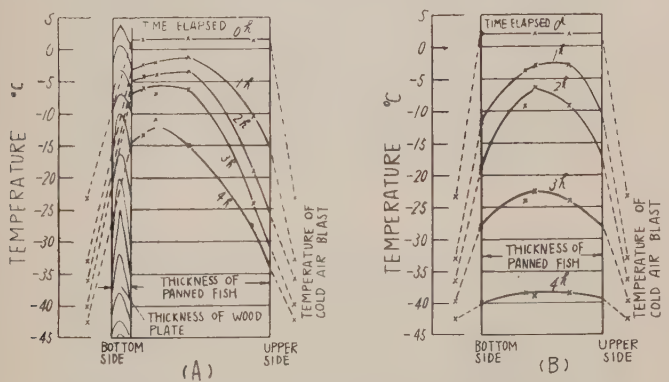
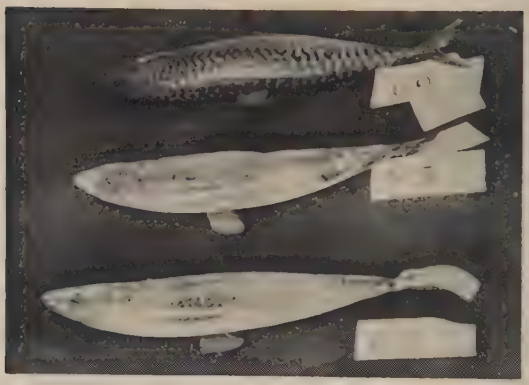


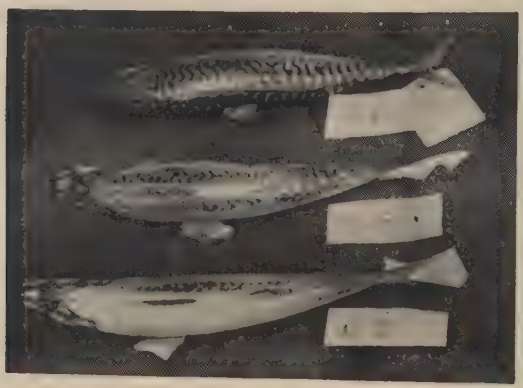
Fig. 10. Temperature distribution in panned fish.
(A) Panned fish in wooden box; (B) Panned fish in ordinary pan.

Generally speaking, the belly side of the fish is slow to freeze compared with the other parts. In Fig. 2 this fact is clearly shown. In this case the albacore is placed across the cold air flow and the belly side toward the air flow. The loss of weight during the freezing rarely exceeded 1% of its original weight and had no tendency that the air-blast increased the weight loss. Some fish such as mackerel changes its skin colour during the freezing as shown in Fig. 11.

This discolouration is greater as the air velocity increases. This was partially prevented by the precooling and freezing of the outer layer of the fish



After freezing



After grazing

After thawing

Fig. 11. Discolouration of fish skin. No. 69 fresh (not frozen); No. 65 precooled; No. 67 not precooled; (Air velocity 4 m/s).

in the still air before the air-blast was applied. Slight discolouration disappeared when the fish was grazed and it regained original colour when it was thawed.

The frozen mackerel, after storing about 1 month at −13°C which is the ordinary storing temperature of frozen food for domestic use in Japan, was grilled and eaten. Among 10 persons who ate the mackerel, 6 could distinguish the fish which was frozen at the air velocity of 4.5 m/s from that frozen in still air, and only one person said that the fish frozen in still air had better taste than the one frozen in the air-blast. The yellowtail frozen in air-blast, was thawed and eaten in raw state after storing 96 days at −13°C. It was delicious and hard to distinguish from the fresh one. When grilled, it also tasted very well.

Snappers frozen in air-blast were grilled and eaten after storing 80 days at −13°C and their tastes were compared with the fresh ones. It was difficult to distinguish the frozen ones from the fresh ones. There is no doubt that fish frozen in the air-blast has better taste than those frozen in still air.

Finally, we wish to express our thanks for the financial aid given by the Fisheries Agency and for the technical aid given by the Kinoshita & Co.

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REFRIGERATION OF FISH ON BOATS IN JAPAN

Kazuo Tanaka

(Received Oct. 15, 1955)

Introduction

One of the remarkable features of many fishing boats in Japan after the World War II is the installation of refrigerating machinery to maintain the freshness of the fish by chilling or freezing until after the fish is unloaded. The demand for such appliances for the pelagic fishing boats is rapidly increasing on account of the fact that more distant fishing grounds are operated on by Japanese fishermen and that larger sized boats have to be used.

Types of fish to be directly refrigerated on boats

The materials to be directly refrigerated on boats are comprised chiefly of Whale-meat, Skip-jack, Tunas as Albacore, Yellow-fin Tuna, Big-eye Tuna, Blue-fin Tuna, Salmon and Flat-fish. Two methods of preservation by refrigeration are employed. One is by chilling and subsequent chilling storage, the other is by freezing and subsequent freezing storage of fish. In the former, the fish is cooled to a low temperature (for instance at 0°C) above its freezing point of −1°C and stored in this condition. No part of the fish will be frozen. On the contrary, in the latter the fish is not only cooled to a very low temperature (for instance at −15°C) below it's freezing point of −1°C but frozen and is stored in that condition. The body fluids of fish will consequently become solid. Such fish as Flat-fish, Skip-jack, Whalemeat and Yellowfin Tuna cannot be retained fresh longer than about 1, 2, 4, 6 weeks respectively by mere chilling storage method. To maintain the freshness of the fish for a longer period as 3, 6 or 12 months the fish must be preserved by the freezing storage method.

In these days, therefore, modern pelagic fishing boats with only a chilling and chilling storage system are attempling to adopt, in addition, the freezing and freezing storage system also.

Kinds of refrigerated ships and their refrigerating machinery

There are three kinds of ships on which refrigerating equipment is provided namely: Refrigerated fishing-boats, Refrigerated mother-ships of fishing boats and Refrigerated fish-carriers. Such small sized fishing-boats as not exceeding 100 gross-tons have comparatively small capacity refrigerators and refrigerated holds or none of them. Medium sized fishing-boats (100∼300 tons) are provided with refrigerating equipment only for chilling and chilling storage of the products. Large sized fishing-boats exceeding 300 tons possess facilities for freezing and freezing storage in addition to regular chilling and chilling storage.

These types of fishing-boats are used almost as Tuna Clippers or Trawlers in this country. There are 1801 ships of the former and 51 of the latter which at present are composed of 1534 and 29 of small size, 218 and 9 of medium size, 49 and 13 of large size of the boats respectively.

The refrigerated mother-ships of fishing-boats are equipped for freezing and freezing storage and they have the comparatively larger capacity equipment for both refrigerating and freezing. For this purpose larger ships are used, i.e., about 3000～10000 tons. Fourteen ships of this size are working at present in Japan.

The refrigerated fish-carriers are provided with refrigerating machinery merely for storage of frozen fish but they have no device for freezing of fish in the boats. Nineteen boats of about 1000 tons are now used as fish-carriers in Japan.

The majority of the refrigerators installed in boats use NH_3 as a refrigerant and it may be considered that the cooling systems are of the direct expansion system for both refrigerated fishing-boats and fish-carriers. On the other hand, the indirect expansion system using $CaCl_2$ brine is used in the refrigerated mother-ships.

Such modern refrigerating machinery as high speed multi-cylinder compressors of NH_3, two stage compressors of NH_3, exceptionally high speed multi-cylinder compressors of F-12, F-22 is used in a few of the Tuna Clippers.

Chilling and freezing of fish

Attention has to be paid to the following items to ensure successful chilling or freezing of fish:—

1. Selecting the raw fish.—The original raw fish to be chilled or frozen must be excellent in quality being as fresh as possible.
2. Preparing the fish.—The fish should be properly prepared before chilling or freezing.

Fish may be treated in following four ways:

a) Round or whole fish i.e., uncut, but sometimes gilled,
b) Semi-dressed fish i.e., eviscerated,
c) Dressed fish i.e., beheaded and gutted, and
d) Filleted fish i.e., fillited.

In Japan, a round or semi-dressed fish is called "Fish body" and a dressed or filleted fish "Fish meat".

The treated fish should be perfectly washed, bled panned and precooled (for freezing only) before being taken into coolers or freezers.

3. Chilling or Freezing the fish.—The fish should be chilled or frozen as quickly as possible.

In chilling, the temperature at the centre of the fish body or fish meat should be brought down to 0°C to +2°C. There are three methods of chilling which are; Air chilling, Brine or Sea-water chilling and Ice chilling. All these methods include the means of chilling storage and the difference of the operation between chilling and chilling storage is not so important in comparison with that of freezing and freezing storage. In air chilling, the fish is cooled by cold air under −2°C and this is the simplest method among the others, therefore this method

of chilling is used in fishing boats below 200 tons. In the larger sized boats, particularly Tuna Clippers, the chilling is effected by NaCl brine or Sea-water under Be 3°, −1°C, so this is widely adopted as the brine or sea-water chilling. The ice chilling is the most popular method in all countries, and is divided into two methods as Dry and Wet systems. In the dry chilling system the fish is chilled by crushed ice (under melting point of 0°C) the ice and fish being in the proportion of 0.8:1 by weight. In the wet chilling system the fish is chilled by sea-water cooled with ice, so this is very similar to the sea-water chilling mentioned above.

The method of freezing of fish is to cool it at least to −10∼−15°C at the central part of the fish body or fish meat so that the body fluid of fish is frozen. There are three methods for freezing of fish which are: Air freezing, Brine freezing and Pressure freezing. In air freezing the fish is cooled by cold air under −25°C and almost 1∼2 M/sec of air velocity. This is generally called "Sharp freezing" or "Semi-air blast freezing". It is a simple and widely practised method but has no superior efficiency. Besides, the desiccation from the fish surface during freezing is high and therefore this method is only used to freeze the round or semi-dressed fish like Tuna. In brine freezing the fish is cooled by NaCl brine under Be 20°, −16°C and almost 0.1∼0.2 M/sec of brine velocity. This is a universal method and has a high level of efficiency in freezing, but it is more complicated to deal with in comparison with the Semi-air blast method. Further, the brine penetrates the fish. This system is also used for freezing the round or semi-dressed Tuna on our fishing boats. In pressure freezing, the fish is cooled by being inserted between cold metal plates in which circulates $CaCl_2$ brine under Be 30°, −26°C in them. It is also generally called "Contact freezing" or "Multi-plate freezing". There are three kinds of mechanism to move the plates up and down. These are (a) the bolts and the nuts, (b) the chain and (c) the oil hydraulic. The freezing time of the fish depends principally upon the condition of contact of the fish surface with the plates and the pressure which may be about 0.14∼0.21 kg/cm²g in all types of the mechanism. Pressure freezing, however, occasionally cracks the fish meat by its high pressure. Most of the refrigerated boats in Japan have adopted this system for high efficiency of freezing, easier operation and compact construction.

The approximate time required for freezing of the fish by these freezers will be 4 to 20 hours and more or less the freezing speed will certainly affect the quality of frozen fish.

4. Handling the frozen fish.—The frozen fish should be fully handled after freezing.

After being taken out of the freezers the fish are de-panned, glazed, wrapped and packaged subsequently. All of these treatments are intended to protect the fish surface from desiccation, oxidation and discolouration during freezing storage.

5. Storage of frozen fish.—The storage temperature of frozen fish should be kept as low as possible.

As a practical standard, the following storage periods of frozen fish for specified storage temperatures may be adopted.

Up to 3 month's storage at −15°C, between 3 and 6 month's storage at −15∼−20°C and up to 12 months or more it should be −20°C or lower.

6. Thawing the frozen fish.—The frozen fish should be thawed as slowly as possible.

The frozen fish cannot be used as it is, it must be suitably thawed before cooking. This procedure, however, is not usually carried out in boats.

Conclusion

In this paper, the principal application of refrigeration on boats for chilling and chilling storage, freezing and freezing storage of the fish as well as the refrigerating machinery installed in the comparatively large type of fishing-boats including mother-ships of fishing-boats and fish-carriers engaged in the pelagic fisheries in Japan was described. But refrigerating devices used at present in these fishing vessels are similar to those used on land. But in view of the special conditions of the boat at sea, proper adaptations to suit the different conditions will have to be employed.

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DEFROSTING OF FROZEN WHALE MEAT

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Introduction

It is a fact that frozen whale meat drips more than frozen fish or cattle meat. The amount of drip depends largely on the freshness of the meat, on the freezing rate, the storage condition and the manner of treatment before or after freezing of the meat, but it is also affected by the defrosting process. The present authors carried out experiments on this point.

Experimental

1. Origin of the Frozen Whale Meat

Experiments were carried out on lean meat of fin whales caught in the Antarctic Ocean and frozen there. The whale, after dissection, was bled and precooled first in air at about 0°C for four hours, and further in sea water at about −0.5°C for two hours, and then cut a rectangular shape of about 12 cm × 30 cm × 39 cm (about 15 kg in weight), put in a freezing pan and frozen on board a refrigerator mother-ship. Two types of freezers are used there: viz. an air freezer (a sort of sharp freezer) and a pressure freezer (a sort of multi-plate freezer). The air temperature and the $CaCl_2$ brine temperature in each freezer was kept at about −19°C and −26°C respectively. The freezing time of the former is about 24 hours, so this is so-called slow freezing, and the latter is about 8 hours, so quick freezing.

2. Defrosting Processes and the Observation on the Defrosted Meat

Defrosting experiments were carried out on frozen meat after three months of cold storage in the refrigarated holds of the ship kept at −20°C or so.

In this connection, there are two types of frozen whale meat, one gives a great deal of quantity of drip showing noticeable contraction, the other does a small quantity of drip showing not any contraction, on thawing. The former was frozen under the condition of the pre-rigor state and has a very good freshness (a high ATP level), this is so-called the contractile whale meat, on the contrary, the latter was frozen under the condition of the post-rigor state and has a not so good freshness (a low ATP level), this is so-called the non-contractile one. Both slow frozen and quick frozen meat used for this experiments is intermediate one of these two types, so this was frozen under the condition of the full-rigor state and showed a slight contraction on thawing, this is so-called the semi-contractile whale meat.

The following defrosting processes were tried on frozen meat, both slow frozen and quick frozen. These were cut a rectangular shape of about 5 cm ×

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10 cm×15 cm (about 0.75 kg in weight) and defrosted. The observations on the defrosted meat are summarized as follows:—

(a) Defrosting in air. Defrosting in still air at room temperature (temp. about +14°C; humd. about 90%) caused slight whitening in the colour of the meat in its surface layer. No damage occurred in the meat tissues, with good palatability. Defrosting time was about 7 hours.

(b) Defrosting in water. The frozen meat was defrosted in running water (temp. about +11°C; speed about 70 cc/sec) from the city main. The defrosted meat had its tissues bruised extensively and its surface quite whitened, the discolouration extending deep into the interior. It tasted quite flat. Defrosting time was about 3 hours.

(c) Defrosting by Joule's heat. 100 volt A.C. was applied to the frozen meat through thin copper sheets on both its sides and defrosted by Joule's heat. Little damage was caused in the tissues and the defrosted meat retained good colour and flavour. If the process can be success, the thawing proceeded fairly uniformly throughout the whole thickness except some partial overheating at the surface. But practically this method is very dangerous. Defrosting time was about 5 minutes.

(d) Defrosting by dielectric heating. A block of frozen meat with thin mica sheets on both its sides was inserted between pole plates and a high frequency electric potential of 14 Mc was applied to the plates to heat the frozen meat dielectrically. The defrosting proceeded nearly evenly throughout the whole thickness, with no bruising in the meat tissue. The defrosted meat retained almost perfectly the bright colour, the elastic firmness and the good palatability of the original raw meat. Defrosting time was about 3 minutes.

3. Quantity of Drip

Quantity of drip during defrosting was measured at regular intervals.

Let W_i and W_f=initial and final weight of the meat under defrosting

w=total quantity of drip

$$\text{and} \quad m=\frac{w}{W_i}, \quad n=1-\frac{W_f}{W_i}$$

then m and n denote the drip ratio and the rate of loss of weight of meat respectively. The difference between m and n, if any, denotes the evaporation due to overheating.

By air defrosting, both slow frozen and quick frozen meat gave practically equal values of m and n (m=0.139 and 0.130 respectively; n=0.141 and 0.136 respectively) while by defrosting by Joule's heat, m was measured to be far lower than n (m=0.002 and 0.102 respectively; n=0.200 and 0.138 respectively) proving a great deal of amount of evaporation of drip due to overheating. It is worthy of notice that quick frozen meat always gives lower values of m and n than the slow frozen.

Defrosting by dielectric heating gave quite small values of m and n as well (m=0.006 and 0.006 respectively; n=0.030 and 0.016 respectively). This fact well agrees with the excellent quality of the defrosted meat by this process. Here also the quick frozen meat gave slightly less m and n than the slow frozen.

A little evaporation by defrosting is suggested by the discrepancy in the values of m and n.

By defrosting in running water, the loss of weight due to thawing is decidedly greater than that by any other process, and sometimes defrosted meat showed the increasing of its weight due to swelling. (m=? and ? as drip was flown away respectively; n=0.202 and −0.018 respectively)

4. Nitrogen Content of Drip

As a measure of the loss of nutritive and flavouring constituents of the meat, total N content as well as the protein N content of the drip were determined by chemical analysis. It was noted that the amount of total N in the drip seems to be affected by the freezing speed, while that of protein N by the process of defrosting. Drip from the slow frozen meat is somewhat richer in its total N content than that from the quick frozen.

The ratio protein N/total N was least with dielectric defrosting. This fact along with the lower amount of drip and evaporation renders dielectric heating the best process of defrosting for the frozen whale meat.

In all cases, the drip just out of the meat was bright red in colour and had no foul odour, with a pH value of 5.8∼6.2.

5. Nitrogen Content of the Defrosted Meat

Soluble N content as well as the soluble coagulable N content in the aqueous extract of the defrosted meat were determined by chemical analysis, as the former follows roughly the quality of the meat as to its palatability and the nutritive constituents, and the latter the degree of denaturation of protein. Dielectric defrosting gave the largest value of soluble N content while the defrosting in water the least, well agreeing with the difference of quality of the defrosted meat by these contrasted two processes. In addition, we can know that not any denaturation due to dielectric current occurred on the defrosted meat by dielectric heating under applying in this degrees.

By comparison of the values of soluble coagulable N/soluble N, it could be concluded that the protein of quick frozen meat is slighly less denatured than that of the slow frozen.

Conclusion

Comperative experiments on the methods of defrosting frozen whale meat which gives an excessive amount of drip on thawing have been carried out. The meat brought from the Antarctic Ocean was defrosted by the following processes, defrosting in air, in water, by Joule's heat and by dielectric heating. In these four ways, the amount of the drip and the nitrogen content in both drip and defrosted meat were measured and the effects of the amount and the nitrogen content of the drip upon the quality of the defrosted meat was studied. It was concluded that the quality of defrosted whale meat depends on the quantity of drip and, though somewhat in less degree, its nitrogen content. In these respects dielectric heating is recommended as an excellent defrosting process for the frozen whale meat, besides it reproduces almost nearly the original quality of raw meat. But air defrosting is none the less practical. As to the effect of freezing process

on the quality of the defrosted meat, quick freezing gives slightly less quantity of drip with less amount of valuable nitrogen than the slow freezing.

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BIOCHEMICAL CONDITION OF WHALEMEAT BEFORE OR AFTER FREEZING AND COLD STORAGE OF FROZEN MEAT

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Introduction

It is a well-known fact that frozen whalemeat yields a large amount of drip on thawing.

In Japan we consume yearly about 20,000 tons of frozen whale meat as food, nevertheless, owing to market conditions, not a small amount of it is compelled to be kept in cold stores for as long as a year or so. Reduction of drip value and check of deterioration of the meat are important problems given to us. Under such circumstances the authors researched into the origin of drip and the changes in the character of frozen meat during the cold storage to obtain some proper measures to reduce the drip value and maintain the high quality of frozen whalemeat.

Experimental

1. Observation on the State of Unfrozen Whalemeat.

It was already reported that three types of whalemeat, i.e. contractile, semi-constractile, and non-contractile, can be observed as its frozen tissues is thawed and that these types seem to be related to the rigor mortis phase of its muscle after death. To investigate into the specific features in the unfrozen condition of these types, changes in pH and weep values were pursued on a block of lean meat cut from the carcass of a fin-whale just after capture in the Antarctic. The test block, measuring about 7 cm×9 cm×15 cm in size, about 1 kg in weight, was kept in still air at +15°C for 4 days for the experiment. The results obtained are shown in Table 1.

Table 1. Change in unfrozen whale meat (at+15°C).

Time Hrs.	0	3	6	9	13	17	22	30	48	61	63	80	96
pH	6.85	6.45	6.30	6.20	6.00	5.80	5.70	5.60	5.50	5.40	5.30	5.38	5.74
Weep %	0.0	0.0	1.0	2.9	7.2	10.5	13.4	14.6	15.5	15.9	16.0	16.0	16.0

The results of observation can be summarized as below:

Pre-rigor state: At its pH value above 6.2, the tissues were observed to be in its pre-rigor stage. It was firm and dry to the touch with bright blackish colour. Little or no weep was exuded from the meat.

Full-rigor state: At pH values between 6.2 and 5.8, the tissues were in progress of rigor and the meat was observed to be fairly hard with rubbery touch and reddish black colour. The weep value was measured at 3~10%.

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Post-rigor state: As the pH value descended below 5.8, the rigor of the tissues was resolved and much weep was exuded from the meat. The meat was featured with its wet and soft touch and dull reddish colour. At pH value of 5.4, the meat began to give off disgustful odour which got intensified as the pH value descended below 5.4, though the meat remained edible even at its lowest pH value 5.3 when the weep value amounted to 16%. Thereafter the pH value turned to rise with no more exudation of weep and the meat turned quite inedible because of its disgustful odour. From these facts it may be presumed that pH value of 5.3 and weep value of 16% are the critical values for the edibility of the meat.

2. Biochemical Change in the Frozen Whalemeat During Cold Storage.

With the view that drip is exuded on thawing from frozen meat due to the contraction of muscle tissues as well as the liberation of water from the muscle protein due to the degeneration of their protein during cold storage, we pursued the change in drip value in relation to the ATP level and the value of $\frac{\text{soluble protein } N}{\text{soluble total } N}$ of the meat, taking former as the index for the contractibility and the latter for the degeneration of protein.

Blocks of meat, both in pre-rigor and post-rigor stage and about 12 cm × 30 cm × 39 cm in size, about 15 kg in weight, were frozen in a multi-plate type freezer by $CaCl_2$ brine of temperature −26°C for 8 hours. The frozen blocks, without being glazed, were packed in cartons and were kept in cold rooms at −8, −16 and −24°C respectively for months. At the end of each definite period of storage, loss of weight of the blocks due to evaporation was measured and then test pieces, about 5 cm × 10 cm × 15 cm in size, about 0.75 kg in weight, were cut from them. Thawed in still air at +20°C, values of pH, drip and $\frac{\text{soluble protein } N}{\text{soluble total } N}$ were measured on the test pieces. With test pieces of pre-rigor type, ATP level (shown by ΔP-P mg/100 g meat) was also measured. The results are given in Table 2.

Remarkable differences were observed between the pre-rigor and post-rigor types. The former showed obviously a feature of thaw rigor with contraction of its tissues, exuding a lot of drip on thawing while the latter showed no appreciable contraction with exudation of comparatively small amount of drip. It was also observed that, during the cold storage, decline in ATP level was noticeable with the pre-rigor, contractile type while decrease in $\frac{\text{soluble protein } N}{\text{soluble total } N}$ was somewhat predominant with the post-rigor, non-contractile one. From these observations it may be accepted that the drip from pre-rigor type is mainly due to the contraction of its tissues by thaw rigor while that from post-rigor one is entirely due to the liberation of water by the degeneration of its muscle protein.

These experimental results lead us to the conclusion that to reduce drip value, regardless of the quality of the meat, whalemeat should be frozen after it is turned to post-rigor type, but in case when the frozen meat is stored at a temperature higher than −15°C or it is to be stored for a long period, to avoid the risk of producing meat with disgustful odour it is better to freeze it in its rigor stage though some increase in drip value is inevitable.

3. Decline of Contractibility at Comparatively High Storage Temperature.

Table 2. Change in frozen whale meat during cold storage.

Storage Temp. °C	Period of Storage Months	Weight Loss in Storage %		pH		*ATP-P* Content mg %		Soluble Protein *N* / Soluble Total *N* × 100 %		Drip in 6 Hrs. %		Drip in 24 Hrs. %	
		C[1]	N[2]	C	N	C	N	C	N	C	N	C	N
−8	0	0.00	0.00	6.50	5.84	51	—	64.0	—	27.9	7.0	31.4	10.2
	1	0.11	0.10	6.18	5.70	33	—	65.8	—	27.3	10.7	30.8	12.4
	2	0.22	0.41	5.99	5.86	27	—	62.9	—	30.6	12.1	33.7	14.4
	3	0.65	0.81	5.99	5.79	26	—	56.7	53.2	33.2	14.0	36.8	18.4
	6	2.28	2.63	5.84	5.95	8	—	53.5	—	3.5	5.6	8.1	9.6
	12	5.21	5.57	5.82	5.52	6	—	55.1	—	6.1	7.7	10.4	11.5
−16	0	0.00	0.00	6.41	5.82	47	—	64.5	74.6	30.8	7.3	33.9	9.2
	1	0.00	0.21	6.25	5.77	55	—	63.9	—	32.2	4.5	34.6	6.0
	2	0.10	0.21	6.23	5.84	30	—	66.5	—	31.5	3.7	34.4	6.4
	3	0.10	0.31	6.09	5.77	27	—	63.4	54.1	33.2	10.1	36.4	14.3
	6	0.51	0.73	6.03	5.90	25	—	65.9	—	24.8	2.2	34.3	5.5
	12	1.84	2.09	6.06	5.68	22	—	60.6	57.9	26.7	6.9	30.3	10.5
−24	0	0.00	0.00	6.21	5.80	43	—	68.1	—	29.4	9.6	31.8	13.6
	1	0.00	0.00	6.15	5.71	51	—	61.9	—	35.2	6.9	36.9	9.6
	2	0.00	0.00	6.13	5.81	38	—	64.2	—	34.8	6.3	36.9	8.9
	3	0.10	0.00	6.12	5.85	30	—	62.9	56.6	36.2	4.9	38.8	10.3
	6	0.20	0.11	6.21	5.92	24	—	65.1	—	20.2	2.5	31.7	6.0
	12	0.51	0.32	6.13	5.89	22	—	70.2	55.0	24.6	12.9	27.7	16.7

C[1]—Contractile type: The samples of this type are frozen at the pre-rigor stage and show contraction on thawing.
N[2]—Non-contractile type: The samples of this type are frozen at the post-rigor stage and show no contraction on thawing.

As is seen in Table 2, meat of contractile type as stored at −8°C showed a marked decrease in its ATP level during the storage and, as its *Δ7P-P* content descended down to 10 mg% or so, the contractibility of the meat vanished while by the storage at −16 or −24°C the *Δ7P-P* content remained above 20 mg% even at the end of 12 month's storage and no such disappearance of contractibility was experienced.

In pursuant to the above observation, experiments were carried out on similar samples frozen at pre-rigor stage to confirm the effect of storage temperature on the contractibility of meat. The test pieces were kept at 0 and −4°C respectively and Table 3 was obtaind. From the Table 3, it proves that meat of contractile type loses its contractibility and shows a marked reduction in its drip value as its *Δ7P-P* content descends to as low as 10 mg% or so, and that the higher is the storage temperature the more rapidly the *Δ7P-P* content decreases.

Table 3. Change in contractile frozen whale meat at moderately high temperature.

Storage Temp. °C	Period of Storage Days	pH	*Δ7P-P* Content mg%	$\frac{\text{Soluble Protein } N}{\text{Soluble Total } N} \times 100$ %	Drip %	
					in 6 Hrs.	in 24 Hrs.
0	0	6.17	35	59.9	20.0	24.2
	1	5.80	8	64.4	7.9	10.3
	2	6.20	4	—	3.3	5.5
	3	6.31	2	66.9	4.2	5.7
−4	0	6.24	48	59.9	29.5	33.2
	1	—	—	—	—	—
	2	—	—	—	—	—
	3	—	—	—	—	—
	15	5.97	20	—	23.3	31.5
	30	5.59	3	58.7	8.4	12.7

Applying the above facts, if we keep the contractile frozen meat at a moderately high temperature, say −2∼−4°C, for several days, we can convert it into non-contractile type and consequently not only the drip value can be reduced but its taste is quite good. As less time is required at higher temperature for the conversion, it is recommended to adopt −2∼−3°C for the treatment to avoid unnecessary deterioration of the meat.

By application of this process to the meat frozen at its pre-rigor stage, reduction of drip and maintenance of high quality of frozen meat can be realized.

The principle of the process may be interpreted as below:

Partial thawing takes place in the frozen meat as it is transferred to a room of some higher temperature than there before. Again, as the velocity of chemical reaction in the body juice is far greater in its liquid state than in its frozen state and greater in the defrosted juice than that before freezing, both the decline in ATP level and the deterioration of the meat may be accelerated

by the partial thawing, but the effect of thawing on ATP is far greater than that on the progress of deterioration as the ATP is a biochemically unstable factor. Thus by keeping the frozen meat at a moderately high temperature for a short period, its contractility fades away rapidly without any appreciable deterioration of the meat. This process may be considered an aging of meat at the frozen state.

4. Proper Storage Temperature of Frozen Whalemeat.

As is seen in Table 2, loss of weight of frozen whalemeat proceeds with the period of storage, and the higher is the storage temperature, the larger is the rate of loss of weight. Hence if we limit the loss of weight by evaporation at 0.5% and take the changes in drip value and deterioration of the meat during cold storage into consideration, the allowable period of storage at a given storage temperature may be provided as Table 4.

Table 4. Allowable period of storage for the storage temperature of frozen whale meat.

Period of Storage Months	Storage Temp. °C
up to 3	−10
6	−15
12	−20

Table 5. Weep, drip and expressible drip.

Storage Temp. °C	Period of Storage Months	Drip[1] %		Weep[2]+Drip %		Expressible Drip[3] %		Weep+Drip+Expressible Drip %	
		C	*N*	*C*	*N*	*C*	*N*	*C*	*N*
−8	0	31.4	10.2	31.4	23.6	11.9	25.2	43.3	48.8
	1	30.8	12.4	30.8	25.8	12.8	21.5	43.6	47.3
	2	33.7	14.4	33.7	27.8	11.8	18.6	45.5	46.4
	3	36.8	18.4	36.8	31.8	13.0	18.2	49.8	50.0
	6	8.1	9.6	8.1	23.0	30.1	19.6	38.2	42.6
	12	10.4	11.5	10.4	24.9	26.9	25.6	37.3	50.5
−16	0	33.9	9.2	33.9	22.6	13.4	20.6	47.3	43.2
	1	34.6	6.0	34.6	19.4	14.0	23.8	48.6	43.2
	2	34.4	6.4	34.4	19.8	15.0	23.4	49.4	43.2
	3	36.4	14.3	36.4	27.7	16.4	22.0	52.8	49.7
	6	34.3	5.5	34.3	18.9	13.9	19.5	48.2	38.4
	12	30.3	10.5	30.3	23.9	15.4	18.5	45.7	42.4
−24	0	31.8	13.6	31.8	27.0	13.2	26.6	45.0	53.6
	1	36.9	9.6	36.9	23.0	13.0	23.8	49.9	46.8
	2	36.9	8.9	36.9	22.3	12.0	21.8	48.9	44.1
	3	38.8	10.3	38.8	23.7	13.0	26.4	51.8	50.1
	6	31.7	6.0	31.7	19.4	11.9	24.0	43.6	43.4
	12	27.7	16.7	27.7	30.1	22.8	23.0	50.5	53.1

1) Drip exuded freely on standing at +20°C for 24 hrs.
2) Weep of contractile type and non-contractile type is taken at 0.0 and 13.4% respectively.
3) Expressible drip under pressure of 2.5 Kg/cm²g after 24 hrs. exudation of drip.

5. Free Water of Whalemeat as Source of Weep and Drip.

The weep value of unfrozen whalemeat is very low at its pre-rigor state and it tends to increase as the meat passes into post-rigor while the drip value is in the reverse order. Nevertheless the sum of the weep and drip values is larger in the contractile type than in the non-contractile. It may be accepted that the liberation of body juice is more vigourous in the resolution of thaw rigor than in that of the rigor mortis in its unfrozen state. Further, if we add up the amounts of weep, drip and the expressible drip under a pressure of 2.5 Kg/cm²g after 24 hrs. liberation of free drip, the total sum remains nearly constant, the average value being at about 0.465, for the both types of meat and through various conditions of storage as well, as is shown in Table 5.

Now if we take the water content of the original whalemeat and that of the drip exuded freely or under pressure roughly at 0.75 and 0.9 respectively, the percentage of the total exuded water can be estimated at $\frac{0.9\times 0.465}{0.75}\times 100=56$ or, roughly speaking, about 56% of the water contained in whalemeat is in the state of free water and the remainder in the form of bound water.

By freezing meat at its pre-rigor state the weep value can be almost nullified and by removing its contractibility before defrosting the drip value can be reduced. Hence the loss of nutritive value and flavour of whalemeat can be minimized.

Conclusion

To obtain frozen whalemeat of biochemically superior quality which exudes small amount of drip and have a good taste on thawing, it is recommended to freeze the meat at its early stage of post-rigor or at its pre-rigor state and remove the contractibility by keeping it in a room of moderate temperature for a short period just before defrosting. Recommended temperature and period of storage for the removal of contractibility are 5 days at −2°C or 10 days at −3°C.

The storage temperature of frozen whalemeat should be kept as low as possible. As a practical standard, allowable storage period at a given storage temperature may be taken as follows: up to 3 month's storage at −10°C, 6 month's at −15°C and 12 month's at −20°C.

Acknowledgement

The authors express their heartfelt gratitude to Mr. I. Gensho, Director of the Tokai Regional Fisheries Research Laboratory, for his kind advices.

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STUDIES ON THE QUALITY INSPECTION OF FROZEN TUNNY IN FROZEN STATE

Sachio HOTANI

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Introduction

The world-wide demand for frozen tunny has been taken in actively, and inspection of its quality is growing now a weighty theme in our daily life and business. Especially for Japan, as an exporter of frozen tunny, this problem is of paramount importance. But unfortunately there has been done no scientific work in this field. Originally, the quality of frozen fish cannot be judged by our sense and its inspection must be performed not only as quickly as possible but without degrading the fish commodity. So, neither examination after thawing nor chemical analysis constitutes an ideal method for inspection, while the drilling test depending on our sense of smell, easily performable as it seems, has been proved to be inferior in respect of accuracy. Lately the author has confirmed that the internal friction of fish meat whether frozen or unfrozen increases with the drop in freshness or with the development of jelly-meat. After this confirmation, the author has proposed a not only simple but accurate method for inspecting the quality of frozen tunny in which he used a sensitive instrument newly he designed so as to estimate any minute change of internal friction of fish body. Many problems relating this inspection method were studied at the same time, after which the author has concluded that by this method it is able to rate the degree of deterioration of the meat or to qualify the so-called "good product".

Experimental method for detecting internal friction of frozen meat

Since, in the case of frozen meat, the increase of internal friction incidental to the fall in freshness is too minute to be measured by any one of known methods, the following technique was elaborated. That is, a thin and slender steel plate acting as a vibrator is plunged into the body of frozen fish down to a definite depth, and made to oscillate by a magnet working at $50\sim$/s. Because the natural frequency of the vibrator has been designed to be also $50\sim$/s, when oscillated as a result of its resonance, the amplitude varies sensitively with the change in internal friction of the meat, making it possible to judge the quality of meat by reading the critical amplitude with a micrometer. Fig. 1 shows an instrument completed for performance of the above technique. This apparatus is to be used as follows: Under oscillating the inserted vibrator (*a*), the magnet (*b*) is moved backwards untill the resonance phenomena stops suddenly, and then the distance thus travelled by the magnet is read on the scale of the micrometer (*c*). The chuck handle (*e*) may facilitate the insertion of the vibrator into the

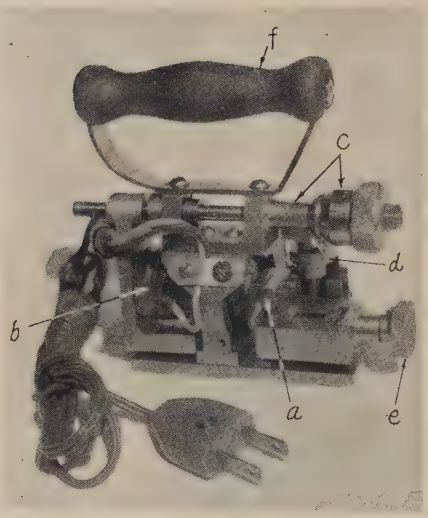

Fig. 1. Apparatus for inspecting the quality of frozen tunny.

meat. (*d*) is a handle for this insertion. For carrying out inspection, this apparatus should be held pressed tightly on the body of frozen tunny by the aid of grip (*f*), the vibrator being inserted in this state.

Experimental results

In order to clarify whether the relation between the internal friction and the rate of freshness of frozen tunny alters from one species to another, some experiments were made using yellowfish, swordfish and albacore. The frozen samples that were different from each other in the grade of freshness were examined their internal friction as given in the smallness of the critical amplitude by the described technique. They were also analyzed of their volatile base content to show the degree of freshness in a customary manner. These results are given in Fig. 2. As seen in Fig. 2 the lower the volatile base content, the larger the critical amplitude. So it may well be concluded that the present method of inspection is quite relevant for discerning the difference in the grade of freshness of so-called "good product" of frozen tunnies.

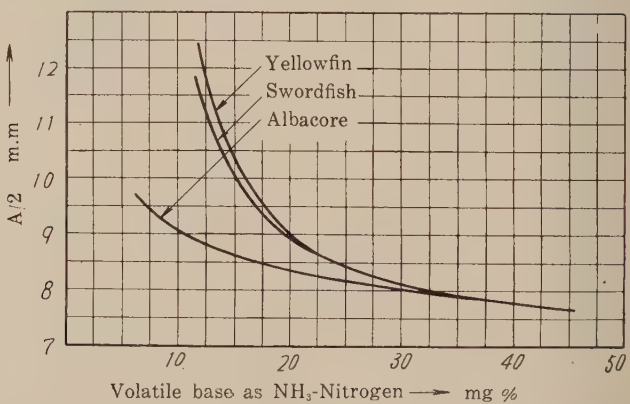

Fig. 2. Relation between amplitude and volatile base as NH_3-nitrogen
A: Internal friction as given by critical amplitude.

In addition to this it is of further interest that at the early stage of deterioration the internal friction of albacore becomes similar to that of swordfish or yellowfin.

Notes:—As for so-called "jellied meat", for a number of experiment the author has thus far done it may be said that jellied meat in middle grade exhibit in general an internal friction equivalent to that of meat whose volatile base has risen up to 25 mg%, both belonging to articles to be rejected.

In order to know the influence of freezing temperature or speed to internal friction of frozen meat, the author carried out some experiments below. Three meat clods from a yellowfin were frozen in still air respectively at −27°C, −16.5°C, and −8°C. Subsequently they were stored equally at −17°C for about two weeks. They were then estimated of their critical amplitude and volatile base strength. By these experiments we may well consider that the difference of freezing temperature or speed has no effect on the internal friction of frozen meat. There is no room for detailed explanation on these results.

Of course, temperature of fish body is dependent on the storage temperature. It is necessary therefore in actual application of this inspection method to know the manner how the temperature of fish body governs its internal friction. Hereupon the author made some experiments on the three samples (albacore) which were stored respectively at −14°C, −17°C, and −20°C. By this result there is detectable no difference in internal friction among these samples in the temperature tested. But with on the frozen fish body that has been kept long at temperatures higher than −10°C and has receded in hardness, it must be award that the value of internal friction of frozen meat tends to become too large to be measured by the present design.

In actural test of frozen products it is far much preferable to work in the corridor than in the cold storage room. From the results of some experiments on swordfish carried out at −9°C after once tested at −17°C, it may be said from their data that this inspection technique can be adopted with success if performable in not too long a time at a relatively higher temperature such as corridor.

Customarily, frozen swordfish is inspected of its quality as filleted, but frozen yellowin and albacore as round. Especially in Japan, all frozen tunnies for export are inspected along such lines. So, in the case of fish frozen as round the vibrator is inserted inevitably through the skin. It is necessary to make clear the effect of mechanical property of skin brought on the result of inspection. For this reason some experiments were carried out. With these samples that were cut out from the back of an albacore and different from each other in the grade of freshness the amplitude of the vibrator kept inserted through the skin on the one hand, and the amount of volatile base contained in the meat located beneath the skin were measured on the other hand. The result of inspection is not altered appreciably at least when obtained on the backside of the fish.

The meat on abdomen side is much rich in oily substance as compared with that on back side, so the internal friction of the former might be found to be much larger too. Considering the case to be tested in round some experiments were made. By this results, with the samples in the same grade of freshness the internal friction is large on abdomen side but small on back side presumably due to the riches in oily substance. Also for the samples from abdomen side, the internal friction in a center is larger than that in a part near the tail. This may

be also due to its large amount of oily substance. From these results, on testing in round by the internal friction method, measuring should be done on the meat in back portion. For the amount and its seasonal variation in oily substance is very minute as compared with the meat on abdomen side.

Finally the author tenders hearty thanks for the Fisheries Agency kindly offered a subsidy to his studies.

Conclusions

Standing on the fact also with the frozen fish the internal friction of meat increases with the fall in freshness, a simple and scientific method of inspection of its quality were devised utilizing the resonance phenomenon of a vibrator inserted into the frozen meat.

By this method, it is possible to grade deteriorated or jellied meat also so-called "good product".

Difference in freezing speed on temperature at the ordinary range does not effect the internal friction. And it is also possible to inspect the articles in corridor of cold storage room.

In inspecting of frozen tunny as round it is adviceable to work with the back of fish.

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A COASTAL SURVEY OF THE GULF OF MEXICO BY JAPANESE FISHING BOATS IN 1936 AND 1937

Hiroshi Niino, Noriyuki Nasu[1] and Robert H. Parker[2]

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While working at the Marine Laboratory of the Texas Game and Fish Commission at Rockport, Texas, the third named author observed a series of charts which had been obtained from the Mexican Government. These charts related to a shrimp fishery survey of the Gulf of Mexico as undertaken by two Japanese boats under contract to the Mexican Government. Upon inspection of the station data for these charts, it was found that extensive bottom notations, bottom and surface water temperatures and other hydrographic information had been included. It was also noted that the area covered by these boats is one on which little information has been published.

These two vessels, the Mino-Maru and Sapporo-Maru sailed from the port of Tobata in Fukuoka, Japan, and surveyed the Mexican-Texas coast from Xalas on the southern side of the Yucatan Peninsula in Mexico to Galveston, Texas. The Mino-Maru took 72 bottom samples in 1936 and the Sapporo-Maru took 144 bottom samples in 1937. According to the station chart many more stations were occupied, and it is quite possible that we do not have the complete station information. Following Tables show the station locations and contain the bottom descriptions.

The depths are divided into five fathom intervals and the number of samples for each interval are given. The ratio of the number of sand sample to the total number of samples (those with descriptions other than sand) are shown in Table 2. Since the present data are insufficient for a more comprehensive discussion, only a few comments on the general character of the sediments in the area covered by the survey is necessary. Generally, the sediments appeared to be coarse close to shore, grading into finer sediments out to about 25 fathoms. At about 30 fathoms the sediments became coarse again, becoming finer from 35 fathoms deeper. This picture is similar to that obtained by Stetson (1953) in his analyses of the sediments of the continental shelf and slope of the western Gulf of Mexico. This gradation from coarse to fine and to coarse again around 30 fathoms, and back to fine again can be observed in Table 2.

There are three factors or combination of factors which can be considered for explaining the occurrence of coarse sediments around 30 fathoms:

1. Dietz in 1952 theorized that there should be wave-cut terraces and remains of littoral and beach deposits at approximately these depths on the continental shelves of the Gulf of Mexico, as a result of the lowered sea-level during the Pleistocene. Dr. Shepord is preparing a

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2) Scripps Institution of Oceanography, University of California.

Table 1. Fishing Report—Minomo Maru Departure—August 27 Arrival—November 8. No. 1. 1936, Sept. 12–22.

Date	Netting Spot Lat.	Long.	Depth of Water (fath.)	Nature of Bottom Sediment
Sept. 12	22—12 N	87—27 W	26	S
13	22—16	89—09	28	S
13	21—40	89—40	13	S
14	19—52	91—03	7	S
14	19—45	91—01	8	S
14	19—59	91—35	18	M
14	18—57	91—38	8	M
15	18—52	91—43	8	M
15	18—55	91—38	8	M
15	19—02	91—43	9	M
15	18—49	91—51	8	M
15	18—53	91—44	7	M
15	18—50	91—35	8	M
16	19—02	91—32	7	M
16	19—12	91—40	12	M
16	19—10	91—31	9	M
16	19—15	91—40	10	M
17	19—20	91—38	16	M
17	19—07	91—45	11	M
17	19—12	92—05	13	M
17	19—12	92—18	19	M
18	19—03	92—24	16	M
18	18—51	92—31	13	M
20	23—02	97—40	19	M
20	23—11	97—38	12	S / Sh
20	23—18	97—42	14	M
20	23—27	97—44	6	M
20	23—39	97—44	8	M
21	24—08	97—19	39	M
21	24—22	97—17	38	M
21	24—35	97—22	25	M
21	24—44	97—10	36	M
21	25—00	97—06	28	M
22	25—14	97—04	28	M
22	25—25	96—56	28	M
22	25—36	96—51	29	M
22	25—38	97—04	8	S / Sh
22	25—47	97—00	12	M

S—Sand; M—Mud; Sh—Shell; R—Rock; Wh S—White Sand; c.l.—clay; Co.—Coral; Gr.—Gravel; M.S.—Mud-sand; ShS—Shell-sand; Small—Small; WS—White sand.

Table 1. No. 2. 1936, Sept. 23 thru Oct. 20.

Date	Netting Spot Lat.	Netting Spot Long.	Depth of Water (fath.)	Nature of Bottom Sediment
Sept. 23	25—34 N	97—05 W	9	M
23	25—20	97—04	19	M
23	25—16	97—16	7	S
23	25—24	97—15	8	M
23	25—09	97—17	12	M
24	25—00	97—10	26	M
24	25—12	97—02	26	M
24	24—47	97—33	6	M
25	22—14	97—33	28	M
Oct. 7	18—15	94—18	18	S
7	18—18	94—11	15	S
7	18—21	94—01	18	Sh, S
7	18—23	93—51	17	M
7	18—45	93—04	20	M
8	18—49	92—51	18	M
8	18—51	92—55	19	M
8	18—58	92—37	14	M
8	19—00	92—29	16	M
8	18—56	92—14	13	M
8	18—56	92—14	13	M
9	19—05	92—03	13	M
9	19—15	91—50	13	M
9	19—14	91—43	11	M
10	19—31	91—11	11	Sh, M
10	19—38	90—58	7	M
11	19—42	91—02	8	M
11	19—53	91—09	11	M
11	19—39	91—10	11	M
14	21—46	87—36	13	S
14	22—13	87—41	22	S
15	22—23	87—50	27	S
15	21—47	87—49	12	S
20	20—11	90—59	7	S
20	20—11	90—49	7	S

paper on sediments of the Gulf of Mexico, shelf, and feels that he should report these results rather than us, His notations in pensil.

2. Coarse materials could be derived from the numerous small calcareous banks or reefs located along the 30 and 40 fathom contours which are described by stetson (1953).
3. Coarse materials could be transported by current action, since it was observed by the third named author that currents existed on the bottom at 40 fathoms strong enough to keep eight-pound lead weights off of

Table 2. Fishing Report—Sapporo Maru Departure: May 3 Tobata Japan Arrival: Sept 5 Balboa. No. 1. 1937, June 22—July 24.

Date	Netting Spot Lat.	Netting Spot Long.	Depth of Water (fath.)	Nature of Bottom Sediment
June 22	22—05 N	87—02 W	21	R
23	21—51	87—25	15	S
23	21—47	87—34	12½	S, R
24	22—16	89—03	27	S, Sh
24	22—19	89—12	28	Whs, c.l.
24	22—38	89—21	33	〃
24	22—50	89—20	47	〃
24	23—00	89—36	77	〃
25	23—02	89—48	88	〃
25	22—29	90—16	44	Co
26	20—20	91—28	17	Whs, c.l.
36	20—12	91—40	20	Whs, c.l.s.
27	20—14	92—03	29	〃
27	20—07	92—13	110	Gr., c.l.
27	20—11	92—04	47	M.S., Sh
28	20—04.5	91—58	27	〃
28	20—04	91—47	24	M
28	20—10.5	91—39	20	〃
28	20—18	91—28	20	〃
28	20—05.5	91—27	19	〃
29	19—54	91—23.5	17½	〃
29	19—45	91—15	14	〃
29	19—30	91—16	12½	〃
29	19—17	91—23	11½	〃
29	19—10	91—34	11	〃
30	18—58	91—40	8	〃
30	18—50	91—34	6	〃
30	18—49	91—42	7	〃
30	18—53	91—55	8½	〃
30	19—07.5	91—55.5	12½	〃
30	19—15	91—45	15	〃
July 1	19—23	91—33	15½	〃
1	19—35	91—23	14½	〃
1	19—45	91—18	15	〃
1	20—03	91—21	15	〃
1	19—55	91—33	20½	〃
2	19—43	91—34	21	〃
2	19—32	91—36	20½	〃
2	19—19	91—42	15	〃
2	19—25	91—53	22	〃
2	19—14	91—50	15	〃
2	19—06	92—00	11	〃

Table 2. No. 2. 1937, July 3—July 24.

Date	Netting Spot Lat.	Long.	Depth of Water (fath.)	Nature of Bottom Sediment
July 3	18—58 N	92—15 E	12	M
3	19—00	92—32	20	〃
3	18—54	92—47	17	〃
3	18—47	92—55	18	〃
3	18—40	93—06	18	〃
4	18—31	93—33	13	M
4	18—33	93—48	24	〃
4	18—34	93—49	29	〃
4	18—20	93—52	6	S
4	18—16	94—05	15	〃
5	18—15	94—17	16	S
5	18—22	94—48	7	〃
5	18—52	95—24	50	M
5	18—50	95—34	25	S
5	18—52.5	95—42	12.5	M
14	23—25	97—42	12	M
15	23—38	97—40	10.5	S
15	24—27	97—39	7	M.S.
15	24—42	97—37	$5^1/_2$	S
15	24—55	97—28	$10^1/_2$	M
16	25—09	97—20	13	M
16	25—22	97—12	11	Sh / M
16	25—36	97—04	12	M
16	25—49	96—58	12	〃
16	26—01	97—03	9	〃
17	26—15	97—03	10.5	Sh / S
17	26—03	97—00	12	ShS / S
17	25—53	96—57	15.5	Sh / M
17	25—43	96—56	19	M
17	25—34	97—03	14	Sh / S
17	25—25	97—09	10	M.S.
18	25—17	97—13	10	M
18	25—09	97—19	10	〃
18	25—02	97—26	10	〃
18	24—51	97—31	9	〃
18	24—49	97—31	10	〃
19	25—02	97—27	10	M
22	21—21	97—17	11	M
22	21—19	97—24	6	ShS / MS
22	21—14	97—23	7	Sh / M
22	21—22	97—20	7.5	〃
23	22—41	97—27	47	M
23	22—53	97—21	100	〃

Table 2. No. 3. 1937, July 27—Aug. 19.

Date	Natting Spot Lat.	Natting Spot Long.	Depth of Water (fath.)	Nature of Bottom Sediment
July 24	25—01	97—23	10.5	M
24	25—13	97—15	11	〃
24	25—21	97—00	11	〃
25	26—34	97—05	17	〃
25	26—47	97—09	16	〃
25	27—03	97—10	14	〃
25	27—16	97—05	15	〃
25	27—28	97—00	14	〃
26	27—42	96—59	11	〃
26	27—50	96—50	12.5	〃
26	28—02	96—40	10	〃
26	28—03	96—25	14	〃
26	28—11	96—16	13	〃
27	28—19	96—05	13	〃
27	28—25	95—52	11	〃
27	28—34	95—41	$9\frac{1}{2}$	〃
27	28—34	95—27	12	〃
27	28—44	94—14	$14\frac{1}{2}$	〃
27	28—48	95—04	12	〃
28	28—55	94—52	11	〃
28	28—57	94—12	$11\frac{1}{2}$	〃
28	28—51	93—59	14	〃
28	28—39	94—01	17	〃
29	28—27	94—03	22	M
29	28—21	94—05	27	M, SH
29	28—08	94—16	32	M
29	27—57	94—23	57	〃
30	27—52	95—45	37	〃
30	27—49	95—59	33	〃
30	27—40	96—05	54	〃
31	26—41	96—35	54	〃
31	26—30	96—36	33	〃
31	26—19	96—32	31	〃
31	26—07	96—32	35	M, S
31	25—56	96—32	36	M
Aug. 1	25—46	96—36	37	〃
1	25—36	96—38	36	〃
1	25—26	96—41	37	〃
1	25—16	96—45	38	〃
1	25—08	96—53	35	〃
2	25—01	97—03	25	〃
2	24—58	97—15	18	〃
2	24—56	97—26	22	〃
3	25—02	97—31	6	〃
3	25—13	97—25	$5\frac{1}{2}$	M, S
3	25—26	97—18	$5\frac{1}{2}$	S

Table 2. No. 4. 1937, Aug. 3—Aug. 19.

Date	Natting Spot Lat.	Long.	Depth of Water (fath.)	Nature of Bottom Sediment
Aug. 3	25—38	97—08	6	small S
4	25—50	97—06	6	M
4	25—05	97—27	$7^1/_2$	〃
4	25—00	97—33	$6^1/_2$	〃
5	25—00	97—31	$6^1/_2$	〃
6	25—12	97—25	$7^1/_2$	〃
6	25—01	97—23	17	〃
6	24—54	97—30	9	S
6	24—46	97—34	6	〃
6	24—34	97—39	$6^1/_2$	〃
18	21—18	86—38	17	WS CO
18	21—30	86—41	17	〃
19	21—44	89—36	17	SH.S CO

Table 3. Ratio of Sand Samples to Total Number of Samples.

Depth of Fathoms	Number of Locations	Percentage of Sand Bottom
5 to 10	60	30
10 to 15	68	18
15 to 20	35	23
20 to 25	12	17
25 to 30	17	41
30 to 35	8	37
35 to 40	9	0
40 to 100	11	36

the bottom. These currents were generally parallel to the shore. However, at the present, very little is known concerning the current systems of the Gulf of Mexico.

Certainly more data are needed to explain this phenomenon, even though the data obtained by the Japanese boats supports the observations made by Stetson.

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PRELIMINARY REPORT ON THE NEW BANK AT THE SOUTHERN END OF EMPEROR SEA MOUNT

Hiroshi Niino and Takeharu Kumagori

(Received Dec. 20, 1955)

Umitakamaru of the training ship of Tokyo University of Fisheries has sailed on her maiden voyage from Tokyo to Hawaiian Island. On 19th September 1955, echo sounding machine of Umitakamura recorded a new bank about 286 m. deep at 35°06.′5N, 171°39.′5E on the surface of a Guyöt which situated between Ojin smit and Kinmai smit near the southern end of Emperor Sea Mount named by Dr. Robert Deitz. This Guyöt has a very extensive flat top and there is a rise of a bank in the center. The diameter of the area surrounded by 1000 m. bathymetric line shows about 20 nautical miles.

The top of the bank is irregular and the bottom character seems rocky one. The outline of the bottom configulation is shown in figure.

Bottom samples were obtained from three stations by snapper. The sample from 35°00.′5N, 171°46.′8E; 600 m. deep is a kind of *Globigerina* ooze which contains *Globigerina*, *Rotalia* and benthonic foraminifera. The samples from the other two station, 300 m. and 600 m. deep, each situated on the steep slope, are belongs to benthonic animals i.e. *Trepella* sp. *Acalcigorgja* sp. *Thourella* sp. *Pseudochonus* sp. An experimental fishing were held on the east side; 35°05.′5N, 171°46.′8E.; on the bank. Many sea birds were flying in the sky and many fishes, *Coryphaea hippurus* Linne, *Prionace glauca* Linne were swimming in the sea.

The data and the result of the experimental fishing are as follows: Number of line: 45; Number of hook in each line: 5; depth of water 43 m; kind of

Fig. 1. The bottom configulation of the new bank.

bait: Makerel pike; atmospheric temperature: 25.6°C; surface water temperature: 25.0°C; 50 m. water temperature: 22.0°C; wind direction: SSW; wind grade 3–4;.

The catches are 1 *Coryphaea hippurus* Linne, 6 *Prionace glauca* Linne, 1 *Xphicus gladius* Linne, 1 *Xphicus* sp.

This bank is situated in the midst of the Pacific ocean and rises from deep sea floor, it is very interesting fact on geology and oceanography. If a detail survey of the bank were finish, it may be possible to solve the geological puzzul of the ocean floor near Emperor Sea Mount. and to get a good fisheries ground.